
8-2013

A Genomic Approach To Identify The Notch Pathway As A Putative Tumor Suppressor In Endometrial Cancer

Rajshi Gandhi

Follow this and additional works at: https://digitalcommons.library.tmc.edu/utgsbs_dissertations



Part of the [Cancer Biology Commons](#), and the [Medicine and Health Sciences Commons](#)

Recommended Citation

Gandhi, Rajshi, "A Genomic Approach To Identify The Notch Pathway As A Putative Tumor Suppressor In Endometrial Cancer" (2013). *Dissertations and Theses (Open Access)*. 394.

https://digitalcommons.library.tmc.edu/utgsbs_dissertations/394

This Thesis (MS) is brought to you for free and open access by the MD Anderson UTHealth Houston Graduate School at DigitalCommons@TMC. It has been accepted for inclusion in Dissertations and Theses (Open Access) by an authorized administrator of DigitalCommons@TMC. For more information, please contact digcommons@library.tmc.edu.

**A GENOMIC APPROACH TO IDENTIFY THE NOTCH PATHWAY
AS A PUTATIVE TUMOR SUPPRESSOR IN ENDOMETRIAL
CANCER**

by

Rajshi Gandhi

APPROVED:

Russell R. Broaddus, M.D., Ph.D., Advisory Committee Chair

Karen H. Lu, M.D.

Rosemarie E. Schmandt, Ph.D.

Kwong K. Wong, Ph.D.

Adrienne S. McCampbell, Ph.D.

APPROVED:

Dean, The University of Texas
Graduate School of Biomedical Sciences at Houston

**A GENOMIC APPROACH TO IDENTIFY THE NOTCH PATHWAY
AS A PUTATIVE TUMOR SUPPRESSOR IN ENDOMETRIAL
CANCER**

A

THESIS

Presented to the Faculty of
The University of Texas
Health Science Center at Houston
and
The University of Texas
MD Anderson Cancer Center
Graduate School of Biomedical Sciences
in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

by

Rajshi Gandhi
Houston, Texas

August 2013

Acknowledgements

A tremendous thank you to my mentor, Dr. Russell R. Broaddus, for his guidance, support, and patience throughout my training. I would especially like to thank him for trusting me and allowing me the independence to learn on my own during this process.

I would like to express my gratitude and appreciation for the guidance and mentorship of my committee members, Drs. Karen Lu, Rosemarie Schmandt, Kwong K. Wong, and Adrienne S. McCampbell. They have greatly shaped my critical and scientific thinking, in addition to offering advice and support to complete this project.

Many thanks to our collaborators at UT-Medical School, especially Dr. Gregory L. Shipley, for their assistance with the qRT-PCR assays and PCR array. Thank you to all members of the Dr. Broaddus lab and Dr. Lu lab, especially Dr. Su-Su Xie, for her help and advice.

A sincere thank you to Dr. Jessica L. Bowser for her guidance, advice, support, and friendship throughout this project.

A Genomic Approach to Identify the Notch Pathway as a Putative Tumor Suppressor in Endometrial Cancer

Publication No. _____

Rajshi Gandhi

Advisory Professor: Russell R. Broaddus, M.D., Ph.D

Endometrial cancer is the most common gynecological malignancy and the fourth most frequently diagnosed cancer among women. The molecular changes that distinguish normal endometrium from endometrial carcinoma are not thoroughly understood. Identification of these changes could potentially aid in identifying at-risk women who are especially prone to develop endometrial cancer, such as obese women and women with Lynch Syndrome.

A microarray analysis was performed using normal endometrium from thin and obese women and cancerous endometrium from obese women. We validated the differential expression of ten genes whose expression was significantly up-regulated or down-regulated using qRT-PCR. All of the genes had distinct expression levels depending on the endometrial carcinoma histotype. As a result, they could serve as molecular markers to distinguish between normal endometrium and endometrial cancer, as well as between low grade endometrial carcinomas and high grade endometrial carcinomas.

Two of the ten genes validated, *HEYL* and *HES1*, are down-stream targets of the Notch signaling pathway. *HEYL* and *HES1* were identified by microarray and qRT-PCR to have a significant decrease in expression in endometrial carcinomas compared to normal endometrium. We further analyzed the differential expression of other components of the

Notch signaling pathway, *Notch4* and *Jagged1*. They were also identified by qRT-PCR to be significantly down-regulated in endometrial carcinomas compared to normal endometrium. Therefore, we believe the Notch signaling pathway to act as a tumor suppressor in endometrial carcinomas.

Table of Contents

Approval Signatures	i
Title page	ii
Acknowledgement	iii
Abstract	iv
Table of Contents	vi
List of Figures	vii
List of Tables	xxi
Chapter 1	1
Introduction	
Chapter 2	14
Materials and Methods	
Chapter 3	20
Gene Validation Results	
Chapter 4	103
Notch Introduction	
Chapter 5	107
Notch Results	
Chapter 6	129
Discussion	
References	140
Vita	147

List of Figures

	Page
Figure 1. Expression of <i>P-cadherin</i> in normal endometrium and in endometrial carcinomas.	22
Figure 2. Expression of <i>P-cadherin</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	23
Figure 3. Expression of <i>P-cadherin</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	23
Figure 4. Expression of <i>P-cadherin</i> in endometrioid endometrial carcinomas (grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	24
Figure 5. Expression of <i>P-cadherin</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	24
Figure 6. Expression of <i>P-cadherin</i> in grade 1 EECs and in grade 3 EECs.	25
Figure 7. Expression of <i>P-cadherin</i> in grade 3 EEC samples and in NEEC samples.	25
Figure 8. Expression of <i>P-cadherin</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	26
Figure 9. Expression of <i>P-cadherin</i> in grade 1 EECs and in grade 3 EECs and NEECs.	26
Figure 10. Expression of <i>P-cadherin</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	27
Figure 11. Expression of <i>P-cadherin</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	27

Figure 12.	Expression of <i>P-cadherin</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	28
Figure 13.	Expression of <i>P-cadherin</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	28
Figure 14.	Expression of <i>P-cadherin</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	29
Figure 15.	Expression of <i>OLFM1</i> in normal endometrium and in endometrial carcinomas.	30
Figure 16.	Expression of <i>OLFM1</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	31
Figure 17.	Expression of <i>OLFM1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	31
Figure 18.	Expression of <i>OLFM1</i> in endometrioid endometrial carcinomas (grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	32
Figure 19.	Expression of <i>OLFM1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	32
Figure 20.	Expression of <i>OLFM1</i> in grade 1 EECs and in grade 3 EECs.	33
Figure 21.	Expression of <i>OLFM1</i> in grade 3 EEC samples and in NEEC samples.	33
Figure 22.	Expression of <i>OLFM1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	34
Figure 23.	Expression of <i>OLFM1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	34

Figure 24.	Expression of <i>OLFM1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	35
Figure 25.	Expression of <i>OLFM1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	35
Figure 26.	Expression of <i>OLFM1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	36
Figure 27.	Expression of <i>OLFM1</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	36
Figure 28.	Expression of <i>VAV3</i> in normal endometrium and in endometrial carcinomas.	38
Figure 29.	Expression of <i>VAV3</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	39
Figure 30.	Expression of <i>VAV3</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	39
Figure 31.	Expression of <i>VAV3</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas (NEECs).	40
Figure 32.	Expression of <i>VAV3</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	40
Figure 33.	Expression of <i>VAV3</i> in normal endometrium and in grades 1 & 2 EECs.	41
Figure 34.	Expression of <i>VAV3</i> in grade 1 EECs and in grade 3 EECs.	41
Figure 35.	Expression of <i>VAV3</i> in grade 3 EEC samples and in NEEC samples.	42
Figure 36.	Expression of <i>VAV3</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	42

Figure 37.	Expression of <i>VAV3</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	43
Figure 38.	Expression of <i>VAV3</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	43
Figure 39.	Expression of <i>VAV3</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	44
Figure 40.	Expression of <i>VAV3</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	44
Figure 41.	Expression of <i>VAV3</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	45
Figure 42.	Expression of <i>SPRY1</i> in normal endometrium and in endometrial carcinomas.	46
Figure 43.	Expression of <i>SPRY1</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	47
Figure 44.	Expression of <i>SPRY1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	47
Figure 45.	Expression of <i>SPRY1</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	48
Figure 46.	Expression of <i>SPRY1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	48
Figure 47.	Expression of <i>SPRY1</i> in grade 1 EECs and in grade 3 EECs.	49
Figure 48.	Expression of <i>SPRY1</i> in grade 3 EEC samples and in NEEC samples.	49

Figure 49.	Expression of <i>SPRY1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	50
Figure 50.	Expression of <i>SPRY1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	50
Figure 51.	Expression of <i>SPRY1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	51
Figure 52.	Expression of <i>SPRY1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	51
Figure 53.	Expression of <i>SPRY1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	52
Figure 54.	Expression of <i>SPRY1</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	52
Figure 55.	Expression of <i>PIK3R1</i> in normal endometrium and in endometrial carcinomas.	54
Figure 56.	Expression of <i>PIK3R1</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	55
Figure 57.	Expression of <i>PIK3R1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	55
Figure 58.	Expression of <i>PIK3R1</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	56
Figure 59.	Expression of <i>PIK3R1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	56
Figure 60.	Expression of <i>PIK3R1</i> in grade 1 EECs and in grade 3 EECs.	57

Figure 61.	Expression of <i>PIK3R1</i> in grade 3 EEC samples and in NEEC samples.	57
Figure 62.	Expression of <i>PIK3R1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	58
Figure 63.	Expression of <i>PIK3R1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	58
Figure 64.	Expression of <i>PIK3R1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	59
Figure 65.	Expression of <i>PIK3R1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	59
Figure 66.	Expression of <i>PIK3R1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	60
Figure 67.	Expression of <i>PIK3R1</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	60
Figure 68.	Expression of <i>PEG3</i> in normal endometrium and in endometrial carcinomas.	62
Figure 69.	Expression of <i>PEG3</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	63
Figure 70.	Expression of <i>PEG3</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	63
Figure 71.	Expression of <i>PEG3</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	64
Figure 72.	Expression of <i>PEG3</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	64

Figure 73.	Expression of <i>PEG3</i> in grade 1 EECs and in grade 3 EECs.	65
Figure 74.	Expression of <i>PEG3</i> in grade 3 EEC samples and in NEEC samples.	65
Figure 75.	Expression of <i>PEG3</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	66
Figure 76.	Expression of <i>PEG3</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	66
Figure 77.	Expression of <i>PEG3</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	67
Figure 78.	Expression of <i>PEG3</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	67
Figure 79.	Expression of <i>PEG3</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	68
Figure 80.	Expression of <i>PEG3</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	68
Figure 81.	Expression of <i>EFNA1</i> in normal endometrium and in endometrial carcinomas.	70
Figure 82.	Expression of <i>EFNA1</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	71
Figure 83.	Expression of <i>EFNA1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	71
Figure 84.	Expression of <i>EFNA1</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	72

Figure 85.	Expression of <i>EFNA1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	72
Figure 86.	Expression of <i>EFNA1</i> in grade 1 EECs and in grade 3 EECs.	73
Figure 87.	Expression of <i>EFNA1</i> in grade 3 EEC samples and in NEEC samples.	73
Figure 88.	Expression of <i>EFNA1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	74
Figure 89.	Expression of <i>EFNA1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	74
Figure 90.	Expression of <i>EFNA1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	75
Figure 91.	Expression of <i>EFNA1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	75
Figure 92.	Expression of <i>EFNA1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	76
Figure 93.	Expression of <i>EFNA1</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	76
Figure 94.	Expression of <i>EDNRA</i> in normal endometrium and in endometrial carcinomas.	78
Figure 95.	Expression of <i>EDNRA</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	79
Figure 96.	Expression of <i>EDNRA</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	79

Figure 97.	Expression of <i>EDNRA</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	80
Figure 98.	Expression of <i>EDNRA</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	80
Figure 99.	Expression of <i>EDNRA</i> in grade 1 EECs and in grade 3 EECs.	81
Figure 100.	Expression of <i>EDNRA</i> in grade 3 EEC samples and in NEEC samples.	81
Figure 101.	Expression of <i>EDNRA</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	82
Figure 102.	Expression of <i>EDNRA</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	82
Figure 103.	Expression of <i>EDNRA</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	83
Figure 104.	Expression of <i>EDNRA</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	83
Figure 105.	Expression of <i>EDNRA</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	84
Figure 106.	Expression of <i>EDNRA</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	84
Figure 107.	Expression of <i>HEYL</i> in normal endometrium and in endometrial carcinomas.	86
Figure 108.	Expression of <i>HEYL</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	87

Figure 109.	Expression of <i>HEYL</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	87
Figure 110.	Expression of <i>HEYL</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	88
Figure 111.	Expression of <i>HEYL</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	88
Figure 112.	Expression of <i>HEYL</i> in grade 1 EECs and in grade 3 EECs.	89
Figure 113.	Expression of <i>HEYL</i> in grade 3 EEC samples and in NEEC samples.	89
Figure 114.	Expression of <i>HEYL</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	90
Figure 115.	Expression of <i>HEYL</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	90
Figure 116.	Expression of <i>HEYL</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	91
Figure 117.	Expression of <i>HEYL</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	91
Figure 118.	Expression of <i>HEYL</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	92
Figure 119.	Expression of <i>HEYL</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	92
Figure 120.	Expression of <i>HES1</i> in normal endometrium and in endometrial carcinomas.	94
Figure 121.	Expression of <i>HES1</i> in normal endometrium and in	

	endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	95
Figure 122.	Expression of <i>HES1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	95
Figure 123.	Expression of <i>HES1</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	96
Figure 124.	Expression of <i>HES1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	96
Figure 125.	Expression of <i>HES1</i> in grade 1 EECs and in grade 3 EECs.	97
Figure 126.	Expression of <i>HES1</i> in grade 3 EEC samples and in NEEC samples.	97
Figure 127.	Expression of <i>HES1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	98
Figure 128.	Expression of <i>HES1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	98
Figure 129.	Expression of <i>HES1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	99
Figure 130.	Expression of <i>HES1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	99
Figure 131.	Expression of <i>HES1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	100
Figure 132.	Expression of <i>HES1</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	100
Figure 133.	Schematic of the Notch Signaling Pathway	106
Figure 134.	Expression of <i>Notch4</i> in normal endometrium and in endometrial	

carcinomas.	112
Figure 135. Expression of <i>Notch4</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3)	113
Figure 136. Expression of <i>Notch4</i> in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29).	113
Figure 137. Expression of <i>Notch4</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	114
Figure 138. Expression of <i>Notch4</i> in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9).	114
Figure 139. Expression of <i>Notch4</i> in grade 1 EECs and in grade 3 EECs.	115
Figure 140. Expression of <i>Notch4</i> in grade 3 EEC samples and in NEEC samples.	115
Figure 141. Expression of <i>Notch4</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	116
Figure 142. Expression of <i>Notch4</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	116
Figure 143. Expression of <i>Notch4</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	117
Figure 144. Expression of <i>Notch4</i> in in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	117
Figure 145. Expression of <i>Notch4</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	118
Figure 146. Expression of <i>Notch4</i> in stages I & II endometrioid endometrial carcinomas compared to higher stages.	118

Figure 147.	Expression of <i>Jagged1</i> in normal endometrium and in endometrial carcinomas.	120
Figure 148.	Expression of <i>Jagged1</i> in normal endometrium and in endometrioid endometrial carcinomas (EECs: grades 1, 2, and 3).	121
Figure 149.	Expression of <i>Jagged1</i> in normal endometrium and in non-endometrioid endometrial carcinomas (NEECs).	121
Figure 150.	Expression of <i>Jagged1</i> in endometrioid endometrial carcinomas (EECs: grades 1, 2, 3) and in non-endometrioid endometrial carcinomas.	122
Figure 151.	Expression of <i>Jagged1</i> in normal endometrium and in grade 1 endometrioid endometrial carcinomas.	122
Figure 152.	Expression of <i>Jagged1</i> in grade 1 EECs and in grade 3 EECs.	123
Figure 153.	Expression of <i>Jagged1</i> in grade 3 EEC samples and in NEEC samples.	123
Figure 154.	Expression of <i>Jagged1</i> in grades 1 & 2 EECs and in grade 3 EECs and NEECs.	124
Figure 155.	Expression of <i>Jagged1</i> in normal endometrium and in endometrial carcinomas by FIGO stage.	124
Figure 156.	Expression of <i>Jagged1</i> in stages IA & IB endometrial carcinomas compared to combined stages II, III, and IV.	125
Figure 157.	Expression of <i>Jagged1</i> in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas.	125
Figure 158.	Expression of <i>Jagged1</i> in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined.	126

Figure 159. Expression of *Jagged1* in stages I & II endometrioid endometrial carcinomas compared to higher stages. **126**

List of Tables

		Page
Table 1.	Typical molecular alterations in Type 1 and 2 endometrial carcinomas.	2
Table 2.	Patient characteristics.	14
Table 3.	Probes and primers for quantitative RT-PCR assays.	16
Table 4.	Function of components in Notch Pathway.	19
Table 5.	Comparisons used to analyze gene expression.	20
Table 6.	Summary of gene validation results.	102
Table 7.	Roche Notch-centric PCR array gene expression levels: normal endometrium vs. grade 1 EECs.	108
Table 8.	Roche Notch-centric PCR array gene expression levels: normal endometrium vs. grade 2 EECs.	109
Table 9.	Roche Notch-centric PCR array gene expression levels: normal endometrium vs. grade 3 EECs.	110
Table 10.	Roche Notch-centric PCR array gene expression levels: normal endometrium vs. NEECs.	111
Table 11.	Summary of qRT-PCR results: Notch pathway components.	128

Chapter 1

General Introduction

Endometrial Cancer

Endometrial cancer (EC) is the most common gynecologic malignancy and the fourth frequently diagnosed cancer among women, with cancers of the breast, lung, and colon being more prevalent (1, 2). In 2012, an estimated 47,130 women were diagnosed with endometrial cancer, with 8,010 deaths resulting from the disease (3).

Endometrial cancer can be differentiated into two broad categories. Type I cancers are of a well-differentiated, endometrioid subtype and comprise about 80% of endometrial cancers. They are low grade (grade 1 or grade 2) endometrioid endometrial adenocarcinomas (EECs) and typically arise in a background of hyperplasia. They are confined to the uterine wall, are minimally invasive, and have a higher survival rate. Additionally, type I cancers are often related to obesity, hormonal imbalance, and hyperlipidemia. Type II cancers are high-grade, poorly differentiated endometrial carcinomas and account for about 20% of ECs. They consist of high grade (grade 3) EECs, malignant mixed Mullerian tumors (MMMT) and papillary serous carcinoma (PSC). These cancers occur in older women, are not associated with high estrogen levels, and have a poor patient prognosis (1, 2). Clear cell carcinomas and undifferentiated carcinomas are also typically considered non-endometrioid tumors.

Cancers generally develop after inactivation of tumor suppressor genes, overexpression of oncogenes, or defects in DNA repair damage. The development of both types of ECs is dependent on different molecular changes. The most common change in type I EC is the inactivation of *PTEN*, a tumor suppressor gene expressed in estrogen-primed environments (1, 2). *PTEN* has been reported to be altered in up to 83% of endometrioid carcinomas and 55% of precancerous lesions (2). Additionally, type I ECs are associated with mutations in *β -catenin* and *K-RAS*, as well as defects in DNA mismatch repair (2). Type II ECs typically exhibit aneuploidy and *p53* mutations (2). Table 1 summarizes the common genetic alterations in endometrial cancer. Due to the differences in the molecular changes of both types of ECs, it is important to further investigate the differential gene expression profiles of various ECs and normal endometrial tissue.

Table 1. Typical molecular alterations in Type 1 and 2 endometrial carcinomas.

Genetic Alteration	Type 1 Carcinomas (%)	Type 2 Carcinoma (%)
<i>PTEN</i> inactivation	50-80 (4)	10 (4)
Microsatellite instability	20-40 (2)	0-5 (2)
<i>β-catenin</i> mutation	20-40 (5)	0-3 (5)
<i>K-RAS</i> mutation	15-30 (5)	0-5 (5)
<i>p53</i> mutation	10-20 (2)	80-90 (2)
HER-2/neu overexpression	10-30 (2)	40-80 (2)
<i>p16</i> inactivation	10 (2)	45 (2)
E-(cadherin) decreased/loss expression	10-20 (2)	60-90 (2)

Prevention of Endometrial Cancer

Cancers such as ovarian and pancreatic cancers have no known precursor lesions and are currently almost impossible to prevent. Endometrial atypical complex hyperplasia is a precancerous condition that coexists with 40% of women who develop endometrial cancer

(6). This risk factor, in combination with Lynch Syndrome and obesity, are the most common factors associated with endometrial cancer development (6).

Lynch Syndrome (LS), an autosomal dominant hereditary syndrome, is the most common form of inherited predisposition to develop cancer (7). Lynch Syndrome results from germline mutations in DNA mismatch repair genes (7). LS patients are at a higher risk of developing certain cancers during their lifetime, including colorectal (52-82%), stomach (6-13%), ovary (4-12%), urinary tract (1-4%), and endometrial (25-60%). However, many patients do not realize they have a family history suggestive of Lynch Syndrome. Thus, it is especially important for physicians to ask about cancers in both maternal and paternal lineages and promote genetic counseling and testing. In the case that women are aware of having Lynch syndrome, they should consider gynecologic screenings and regular check-ups (6).

Estrogen and progesterone are known to be responsible for endometrial cell proliferation. Due to the conversion of androstenedione to estrone and the aromatization of androgens to estradiol in peripheral adipose tissue, obese women have higher levels of endogenous estrogen than those at a healthy weight (8). Risk for endometrial cancer has been correlated with these high serum estrogen levels. Therefore, obesity is one of the biggest risk factors for endometrial cancer development. An estimated 90% of type 1 EC patients are obese. Additionally, many EC patients typically develop obesity-driven comorbidities, such as type II diabetes, hypertension, and pulmonary disease (8). It is extremely important to initiate corrective interventions such as diet, exercise or even bariatric surgery. If weight loss is difficult, patients should also consider hormonal

interventions, such as oral contraceptive pills, in order to regulate menstrual cycles to help prevent endometrial hyperplasia or cancer.

Not all at-risk women will develop EC. Identification of molecular changes that distinguish normal endometrium from endometrial cancer could potentially aid in identifying at-risk women who are especially prone to develop endometrial cancer. Therefore, it is especially important to identify tissue biomarkers that will help to indicate an individual woman's predisposition to EC.

Microarray Analysis

A microarray analysis was performed using normal proliferative-phase endometrium from thin and obese women and cancerous endometrium from obese women. This array provided 22 candidate genes that could help distinguish obese normal endometrium from obese endometrial cancers. Of the 22 candidate genes, ten genes that had not been previously studied in depth in endometrial cancer were chosen for validation using quantitative real time polymerase chain reaction (qRT-PCR). Some background on these genes is provided below.

Placental Cadherin (P-Cadherin)

Placental cadherin (P-cadherin) is part of the classical cadherin family, which are calcium dependent cell adhesion proteins that contribute to embryogenesis, homeostasis of normal epithelia and tumorigenesis (9). P-cadherin is found in the basal layers of stratified epithelia, suggesting that it functions in cell differentiation and growth (10).

Van Marck et al. (9) examined the specific function of P-cadherin in colon and bladder carcinomas through immunohistochemistry. They determined that a decrease or loss of membranous P-cadherin expression significantly correlated with a higher tumor grade and could distinguish well differentiated tumors from moderately or poorly differentiated colorectal cancers. Their results suggested that P-cadherin has anti-invasive and anti-migratory functions (9).

As P-cadherin could have a prognostic significance, Stefannson et al. (10) investigated the expression patterns of cellular adhesion markers, including E-cadherin, P-cadherin, and β -catenin, in a series of endometrial carcinomas. They collected tumor tissues from 286 patients and recorded histologic characteristics such as histotype, FIGO grade, solid growth, necrosis, myometrial infiltration, and growth pattern.

Increased P-cadherin expression was seen in 83 of the patients, with a significantly higher amount of clear cell or serous papillary type. Increased P-cadherin expression also correlated with vascular invasion, deep myometrial invasion, and increasing FIGO stage. Specifically among the endometrioid tumors, high P-cadherin expression correlated with increasing FIGO grade and deep myometrial invasion. This group's findings imply that P-cadherin up-regulation is important for distinction between endometrioid and non-endometrioid tumors (10).

OLFM1 (Noelin-1)

OLFM1, also called *Noelin-1*, encodes a secreted glycoprotein belonging to a family of olfactomedin domain containing proteins. It functions in regulating the production of neural crest cells by the neural tube and is down-regulated in endometrial cancer, Ewing's

sarcoma, and neuroblastoma (11). In a study performed by Wu et al. (11), strong cytoplasmic staining of OLFM1 was seen in patients with non-small cell lung cancer, especially in adenocarcinomas compared to normal lung tissues. However, they did not find a difference in expression of OLFM1 in adenocarcinomas at early stage and late stage.

Wong et al. (12) recently used gene expression profiling to identify potential molecular markers in endometrial cancer. Genome-wide expression profiles were determined for 84 endometrioid endometrial tumors and normal samples. Their initial microarray found *OLFM1* to be one of the genes down-regulated in endometrial cancers compared to normal endometrium. Further qRT-PCR was performed on a different set of 56 cancer and 29 normal samples. The change in expression seen by qRT-PCR was comparable to the initial microarray results. Their findings identified potential clinical markers for endometrioid endometrial cancers (12).

VAV3

VAV3 encodes a guanine nucleotide exchange factor (GEF) for Rho family GTPases, and it is part of the Vav family proteins, of which there are three. The levels of each of the three proteins vary depending on the tissue examined; hematopoietic cells mainly express VAV1 while VAV2 and VAV3 are ubiquitously expressed. Activation of Vav proteins occurs through tyrosine phosphorylation by receptor protein tyrosine kinases or cytoplasmic protein tyrosine kinases (13).

In breast cancer, VAV3 binds to estrogen receptor alpha (ER α), enhancing its activity through the PI3K-AKT signaling pathway. This potentiates epidermal growth factor (EGF) for cell growth and ER α activation. Lee et al. (13) detected VAV3 staining in 67% of

the well to moderately differentiated breast cancer samples analyzed and 100% of the poorly differentiated breast cancer samples analyzed. Additionally, decreased VAV3 expression significantly inhibits both estrogen independent and estrogen dependent growth in breast cancer cells (13). Overall, they determined that VAV3 overexpression increases ER α -mediated signaling and contributes to the progression of breast cancer (13).

Sprouty1 (SPRY1)

Sprouty proteins were first discovered as negative regulators of fibroblast growth factor (FGF) signaling during tracheal and eye development in *Drosophila* (14). They were later established as inhibitors of growth factor induced receptor tyrosine kinase (RTK) signaling pathways that take part in *Drosophila* development. *Drosophila* has only one *Spry* gene while humans and mice have at least four *Spry* homologs, *Spry1-4* (15).

Mammalian Sprouty proteins block growth factor induced cell responses through inhibiting the RTK dependent RAS/mitogen activated protein kinase signaling pathways. Many mechanisms have been proposed for *Spry* inhibition of this pathway, such as inhibition of Raf (15). This pathway plays a role in tissue remodeling. *SPRY1* and *SPRY2* are specifically expressed in the luminal epithelial cells of the normal breast ducts. Increased expression is seen during tissue remodeling as the epithelial ducts are branching. *SPRY1* and *SPRY2* are consistently down-regulated in breast cancers. This suggests Sprouty proteins act as regulators of epithelial cell growth (16).

In prostate cancer, the *Spry1* homolog is down-regulated in prostate cancer tissues compared to normal prostate tissue, as measured by qRT-PCR. In addition, an increased expression of *SPRY1* inhibits prostate cancer cell proliferation (15).

PIK3R1

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that have significant regulatory roles in cell survival, differentiation, and proliferation. Depending on their substrate characteristics and substrate specificity, they are separated into three classes. The Class I enzymes are more commonly known; they are subcategorized into Class IA PI3Ks and Class IB PI3Ks. Class IA PI3Ks are activated by G-protein coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), and various oncogenes, such as RAS while GPCRs are the sole regulator of Class IB enzymes. Class IA PI3Ks are heterodimers consisting of a p85 regulatory subunit and a p110 catalytic subunit. The PI3K regulatory subunit 1 (PIK3R1) encodes p85 α . Genomic analyses of human glioblastomas found *PIK3R1* to be mutated in 10% of tumors analyzed and somatic mutations of *PIK3R1* have also been seen in primary human ovarian and colon tumors (17).

The PI3K pathway is mutated in several cancer lineages, including endometrial cancer. Mills et al. determined that *PIK3R1* is frequently mutated in 20% of endometrial cancers, higher than any other cancer lineage which suggests selective targeting in EC. Co-mutations in other PI3K pathway members may also contribute to cell transformation. Co-mutations in the PI3K pathway occur more frequently in EC than in other cancer types, with *PIK3CA*, *PIK3R1*, or *PIK3R2* mutations being more common when the PTEN protein is retained. Additionally, Mills et al. reported haploinsufficiency of *PIK3R1* can result in PI3K pathway activation, whereas homozygous deletion inhibits the pathway. Overall, there are several mechanisms through which *PIK3R1* aberrations can contribute to tumorigenesis, but further studies are required to elucidate the effects of each mutation (18).

PEG3

PEG3, which is highly conserved between mice and humans and thus likely contributes to critical cellular functions, has 12 Kruppel-type zinc finger DNA binding domains and a protein-protein interaction domain (19). *PEG3* possibly acts as a transcription factor due to its two proline- and acidic amino acid-rich regions which have transactivation domains. Also, *PEG3* potentially plays a role in the p53/c-myc-mediated apoptosis pathway, suggesting it functions in carcinogenesis. While the highest *PEG3* mRNA levels have been found in the placenta, ovary, uterus, testis, and brain, *PEG3* is expressed nearly universally (19). Decreased *PEG3* expression levels have been observed in endometrial and ovarian cancer through gene expression profiling (19). Additionally, biallelic silencing of *PEG3* has been reported in human glioma cell lines (19).

Dowdy et al. (19) further investigated the possible tumor suppressive function of *PEG3* in gynecologic cancers. Through qRT-PCR and methylation specific PCR, they detected *PEG3* expression in normal ovarian, cervical, endometrial, and placental tissues, but they did not observe *PEG3* expression in different endometrial (Ishikawa, AN3CA, KLE, RL95-2, HEC-1A, HEC-1B, ARK-1, ARK-2) and cervical cancer cell lines. They also determined *PEG3* expression to be inversely correlated with promoter methylation levels, suggesting *PEG3* expression is controlled by epigenetic mechanisms.

Ephrin-A1 (EFNA1)

The first ephrin protein *Ephrin-A1*, or *EFNA1*, was discovered in 1990 as an early response gene to TNF- α stimulation in human umbilical vein endothelial cells. It was later identified as a ligand for the EphA2 receptor. The ephrin family is composed of eight

members, which are further divided into A and B subgroups. Ephrin ligands are separated based on their method of cell membrane attachment while ephrin receptors are divided depending on their sequence homology. Generally, EphA receptors bind to ephrin-A ligands and EphB receptors bind to ephrin-B ligands (20). Expression of the ligands and receptors simultaneously may cause autocrine or paracrine signaling in tumor cells. It may also result in both forward and reverse signaling which contributes to the development and progression of the tumor (21).

Altered ephrin expression has been reported in several human cancers, such as melanomas, carcinomas, sarcomas, and brain tumors. Some tumors have an elevated expression of ephrins while others show a decreased expression. Literature has shown ephrins to contribute to tumorigenicity, angiogenesis, metastasis, and invasion and migration (21). In addition, studies have reported that a higher expression of *EFNA1* has been associated with poorer patient survival in various cancers, including ovarian cancers, melanomas, and vulvar carcinomas (21).

EphA2, the receptor for *EFNA1*, is up-regulated in many solid tumors, including breast, prostate and non-small cell lung cancer. In addition, EphA2 plays a role in tumor angiogenesis. Merritt et al. (22) investigated EphA2 in endometrial cancer. Overexpression of EphA2 is associated with up-regulation of angiogenic markers, such as vascular epithelial growth factor (VEGF) and microvessel density. VEGF and microvessel density are also associated with high-grade, high-stage phenotypic features of endometrial cancers. Due to the strong correlation of EphA2 with angiogenesis, Merritt et al. (22) also investigated the therapeutic implications of EphA2 by using an EphA2 agonistic antibody, EA5, in uterine cancer models. EA5 binds to EphA2, causing it to be phosphorylated and thus, decreasing

EphA2 expression. Therefore, EA5, along with cytotoxic therapy, significantly reduced the growth of endometrial cancers (22).

Endothelin receptor type A (EDNRA)

EDNRA, also known as endothelin receptor type A (ET_AR), is a distinct subtype of G-protein coupled receptors, acting on endothelin-1 (ET-1). This ligand-receptor complex is overexpressed in primary and metastatic ovarian carcinomas. Through EDNRA, ET-1 functions as an autocrine growth, survival, and angiogenic factor specifically in ovarian tumor cells. In doing so, it activates several signaling pathways such as the mitogen activated protein kinase (MAPK) pathway and the PI3K-Akt pathway. Rosanò et al. (23) have shown that through the activation of the ET-1/EDNRA pathway, ovarian cancer cells undergo an epithelial-to-mesenchymal transition. Additionally, EDNRA expression was significantly associated with a decrease in *E-cadherin*, which is responsible for mediating cell-cell interaction. Grades 3 and 4 ovarian serous and endometrioid carcinomas showed higher expression levels compared to early grade cancers, suggesting EDNRA expression is grade dependent. In addition, when ET-1/EDNRA signaling was interrupted through EDNRA antagonists, *E-cadherin* transcription levels increased and tumor growth decreased. This data suggest EDNRA contributes to the epithelial to mesenchymal transition (23).

HEYL

HEYL belongs to the hairy enhancer of split related family of genes and is a basic helix-loop-helix transcription factor. There are three related HEY proteins, HEY1, HEY2, and HEYL (24). HEYL can be activated through constitutive active forms of Notch

receptors (25). Its expression in human endothelial cells is correlated with increased cell growth and anti-apoptosis activity (25). In breast cancer, Parker et al. (25) have found it to be overexpressed. A *HEYL* mixture probe was used to test for the expression of *HEYL* in normal tissue and invasive ductal carcinomas. They did not find any staining in the normal tissues, but there was a strong labeling of the *HEYL* probes in invasive ductal carcinomas. Their study showed a dramatic induction in breast tumor vasculature by at least 10 fold relative to normal breast vasculature. Additionally, they found a ≥ 10 -fold increase in breast tumor endothelial cells. Specifically, in invasive breast cancer, *HEYL* expression was predominantly seen in breast endothelial cells with minimal expression in tumor epithelial cells (25).

HES1

HES1 is part of the *Hes* family, which also belongs to the basic helix-loop-helix family of transcription factors. *HES1* is one of the target genes of the Notch signaling pathway, which is involved in many different cancers (26).

Zhang et al. (26) investigated the role of Notch and *HES1* in osteosarcoma invasion and metastasis. They used RT-PCR to analyze human osteosarcoma cell lines. They determined that Notch and *HES1* expression correlate with the metastatic phenotype in osteosarcoma cells. Additionally, *HES1* expression has also been reported to be higher in ovarian carcinomas compared to benign ovarian tumors (27).

Gotte et al. (28) investigated the effects of Musashi-1, a stem cell marker, on endometrial carcinoma progression and apoptosis through the Notch signaling pathway. While the specific function of Musashi-1 in endometrial cancer is unknown, other studies

have found it to participate in tumor growth. Typically, Musashi-1 acts as positive regulator of Notch mediated transcription by repressing Numb proteins, which induce Notch internalization and intracellular degradation. Gotte et al. (28) observed the effects of Musashi-1 knockdown on protein expression of vital components of the Notch signaling pathway in the endometrial adenocarcinoma cell line, Ishikawa. Knockdown of Musashi-1 resulted in a significant down-regulation of Notch1 and *HES1* in Ishikawa cells.

Chapter 2

Materials and Methods

Human normal endometrial biopsies, tumor samples, and cell lines.

Following IRB approval (LAB01-718), frozen endometrial carcinomas (n=78) and benign endometrium (n=10) were obtained as residual tissues from hysterectomies submitted to the Department of Pathology, M.D. Anderson Cancer Center. Benign endometrial tissues were typically obtained from hysterectomies for cervix dysplasia/cancer uterine leiomyomas, or benign ovarian cysts. Characteristics of the patients whose specimens were used are summarized in Table 2.

Table 2. Patient Characteristics.

Normal Endometrial Samples	n = 10
Age (years)	
Mean	46.7
Median	46
Body Mass Index (kg/m ²)	
Mean	27.8
Median	27.5
Endometrial Cancer Samples	n = 78
Age at diagnosis (years)	
Mean	62.8
Median	63
Body Mass Index (kg/m ²)	
Mean	33.7
Median	31.25
Stage at diagnosis, n (%)	
I	48 (61.5)
II	5 (0.06)
III	21 (26.9)
IV	4 (0.05)
Histology, n (%)	

Endometrioid	49 (62.8)
Non-endometrioid	29 (37.2)
Serous and Mixed ¹	15 (19.2)
MMMT ¹	14 (17.9)
Endometrioid grade, n (%)	
1	9 (18.4)
2	22 (44.9)
3	18 (36.7)

¹Tumors with mixed endometrioid and non-endometrioid components.

Surgical stage was determined using the 2010 FIGO staging system. Stage IA and IB tumors are confined to the uterus, with IA tumors having none or less than half myometrial invasion and IB tumors having more than half myometrial invasion. Stage II tumors are also confined to the uterus, but they invade cervical stroma. Stage IIIA tumors invade the uterine serosa, ovaries or fallopian tubes, and Stage IIIB tumors involve the vagina and the parametrial area. Stage IIIC1 tumors have pelvic lymph node involvement, and Stage IIIC2 tumors have para-aortic lymph node involvement. Stage IVA tumors invade the bladder and the bowel mucosa, while stage IVB tumors have distant metastases including abdominal metastases and the inguinal lymph nodes.

RNA Isolation

Total RNA was isolated from tissues using a phenol-based method. Samples were homogenized via a polytron in TRIzol® Reagent (Invitrogen, Carlsbad, CA) and mixed with chloroform. Lysates were separated into three phases by centrifugation. Total RNA was precipitated from the aqueous phase with isopropanol, washed with 75% DEPC-ethanol, and re-suspended in DEPC H₂O. Isolated RNA was DNase (Epicenter Biotechnologies, Madison, WI) treated.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Probe-based, real-time quantitative real-time PCR (qRT-PCR) assays for *P-Cadherin*, *OLFM1*, *VAV3*, *SPRY1*, *PIK3R1*, *EDNRA*, *EFNA1*, *PEG3*, *HES1*, *HEYL*, *Notch4*, *Jagged1*, and *18S rRNA* were developed using Primer Express software (Applied Biosystems) based on sequences from Genbank. qRT-PCR was performed using a 7700 Sequence Detector (Applied Biosystem, Foster City, CA). All qRT-PCR reactions were performed in the Quantitative Genomics Core Laboratory (UT-Houston Medical School, Houston, TX). Primer and probe sequences and accession number of assays developed and used in this thesis are indicated in Table 3.

Table 3. Probes and primers for quantitative RT-PCR assays.

Transcript	Taqman Primers and Probe	Accession Number
<i>P-Cadherin</i>	1820(+) CCAGGTACTTCTGTGATG 1879(-)GTAGGTGTAGATGGCATC 1860(-) FAM- TCCTCATCCGTGGCTGTCA-BHQ1	NM_001793
<i>OLFM1</i>	705(+) GAGAAGGTGCAGAACATG 773(-) GTACTGCAAGTCTCTCTG 752(-) FAM-CCGCCTGTCCAAGACCTCTATG-BHQ1	NM_014279
<i>VAV3</i>	2320(+) AGAACCTTAGATACAACCTCTG 2401(-) TGTTGCCTGCTCTATTAC 2348(+)FAM-CATACAAGGAGCCAGAACATTCAGC-BHQ1	NM_006113

<i>SPRY1</i>	995(+) GTCAAGGGCATCTTCTAC 1068(-) GAACAGGAGCAAGGATTA 1015(+) FAM-CTGCTCCAATGACGACGAAGG-BHQ1	NM_005841
<i>PIK3R1</i>	2710(+) CAATGTCACACTAGCCTA 2782(-) CAGGAGAAGGATCAAAGA 1756(-) FAM-TTCATCGCCTCTGCTGTGCBHQ1	NM_181523
<i>HES1</i>	417(+) CTGATTTTGGATGCTCTG 500(-) GTGCTTCACTGTCATTTC 478(-) FAM-ATGTCCGCCTTCTCCAGC-BHQ1	NM_005524
<i>EDNRA</i>	1502(+) AAGCCGTATATTGAAGAAAAC 1579(-) ATGAGCAGTAAGAACTAAGTA 1552(-)FAM-CATCGGTTCTTGTCCATCTCGTTAT_BHQ1	NM_001957
<i>PEG3</i>	3813(+) GTACACACATTCTGTAATTCA 3897(-) CACTTTGGACATTCATACAG 3835(-)FAM-CCATTCCATCAGCGAGTATCAGAGA-BHQ1	NM_006210
<i>HEYL</i>	1132(+) ACTGGCTCATATGTGAAG 1208(-) CAGTGAGAAGGAGAGATG 1153(+) FAM-CGTTCTCCAGCCATCAGAGG-BHQ1	NM_014571
<i>EFNA1</i>	415(+) CTGTCTGAGAAGTTCCAG 495(-) GGAGATGTAGTAGTAGCTG 473(-)FAM-CCTTCTTTGAACTCCTTGCCCAG-BHQ1	NM_004428

<i>Jagged1</i>	4150(+) AACCGAATGGAGTACATC 4239(-) GACACGACAGTTTAAAGAA 4210(-) FAM-TCAGACTCTACCTAGCGGCG-BHQ1	NM_000214
<i>Notch4</i>	897(+) CAGAGAAAGACTCCACCT 979(-) ACAGTTGTCTGGATTAC 915(+) FAM-TTCACCTCTGCCTCTGTCC-BHQ1	NM_004557

Roche PCR Array

A Notch-centric PCR array (Roche Molecular Systems, Branchburg, New Jersey) was used to screen the components of the Notch signaling pathway. A PCR array combines the gene expression profiling technique of a microarray and the quantitative aspect of qRT-PCR. 5 µL of RNA isolated from 72 normal (n = 9) and endometrial carcinoma samples (n = 63) were added to a 96-well plate. Then, 15 µL of Roche Master Mix was added to the wells and mixed. qRT-PCR reactions were performed to test for *DLL1*, *DTX1*, *JAG1*, *JAG2*, *ADAM10*, *PSEN1*, *PSEN2*, *PSENEN*, *NOTCH1*, *NOTCH2*, *NOTCH3*, *NOTCH4*, *ATP5A1*, *TFRC*, *TBP*, and *YWHAZ* expression. Table 4 summarizes the function of these components of the Notch pathway.

Table 4. Function of Components in Notch Pathway.

Genes	Function in Notch Pathway
<i>DLL1</i>	Ligand (Notch binding)
<i>DTX1</i>	Ligand (Notch binding)
<i>JAG1</i>	Ligand (Notch binding)

<i>JAG2</i>	Ligand (Notch binding)
<i>ADAM10</i>	Enzyme that cleaves ligand-receptor complex
<i>PSEN1</i>	Notch receptor processing
<i>PSEN2</i>	Notch receptor processing
<i>PSENEN</i>	Notch receptor processing
<i>NOTCH1</i>	Notch Receptor
<i>NOTCH2</i>	Notch Receptor
<i>NOTCH3</i>	Notch Receptor
<i>NOTCH4</i>	Notch Receptor
<i>ATP5A1</i>	Data normalization
<i>TFRC</i>	Data normalization
<i>TBP</i>	Data normalization
<i>YWHAZ</i>	Data normalization

Statistical Analysis

Statistical differences were calculated using the Mann-Whitney U test and the ANOVA test. The Tukey test was performed for multiple comparisons. Differences were considered significant if $p < 0.05$.

Chapter 3

Gene Validation Results

The expression of each gene validated was analyzed according to tumor histotype, tumor grade and surgical stage. A complete list of comparisons is provided in Table 5.

Table 5. Comparisons used to analyze gene expression.

Grade
Normal vs. Endometrioid
Normal vs. Non-endometrioid
Normal vs. Grade 1 Endometrioid
Endometrioid vs. Non-endometrioid
Grade 1 Endometrioid vs. Grade 3 Endometrioid
Grade 3 Endometrioid vs. Non-endometrioid
Grade 1 and 2 Endometrioid vs. Grade 3 Endometrioid and Non-endometrioid
Stage
For all tumors: Stages IA and IB vs. Stages II, III, IV
For all tumors: Stages I and II vs. Stages III and IV
For endometrioid tumors only: Stages IA and IB vs. Stages II, III, IV
For endometrioid tumors only: Stages I and II vs. Stages III and IV

The rationale for some of these group comparisons is provided below.

1. Normal vs. Grade 1 endometrioid – Grade 1 endometrioid tumors are well-differentiated. In the past, our laboratory has observed that for many genes and pathways, there is little difference between normal endometrium and grade 1 endometrioid tumors.
2. Endometrioid vs. non-endometrioid – Endometrioid tumors typically have a better patient prognosis than non-endometrioid tumors.
3. Grade 1 endometrioid vs. Grade 3 endometrioid – This represents a comparison of well-differentiated endometrioid tumors (grade 1) and poorly differentiated endometrioid tumors (grade 3).
4. Grade 3 endometrioid vs. non-endometrioid – Some authorities have posed the possibility that grade 3 endometrioid tumors are more like non-endometrioid tumors. It is known that a substantial subset of grade 3 tumors has *p53* mutations. Mutation of this gene is characteristic of the non-endometrioid carcinomas.
5. Grades 1 and 2 endometrioid vs. grade 3 endometrioid and non-endometrioid – This represents a variation of the endometrioid-non-endometrioid comparison. In this case, the grade 3 tumors are combined with the non-endometrioid category.
6. Stages IA and IB vs. Stages II, III, IV – Patients with higher stage endometrioid tumors typically receive additional treatment (radiation treatment and/or chemotherapy) following surgery.
7. Stages I and II vs. Stages III and IV – Patients with stage I or stage II tumors have tumor confined to the uterus. Stages III and IV mean tumor is found outside the uterus.

P-cadherin

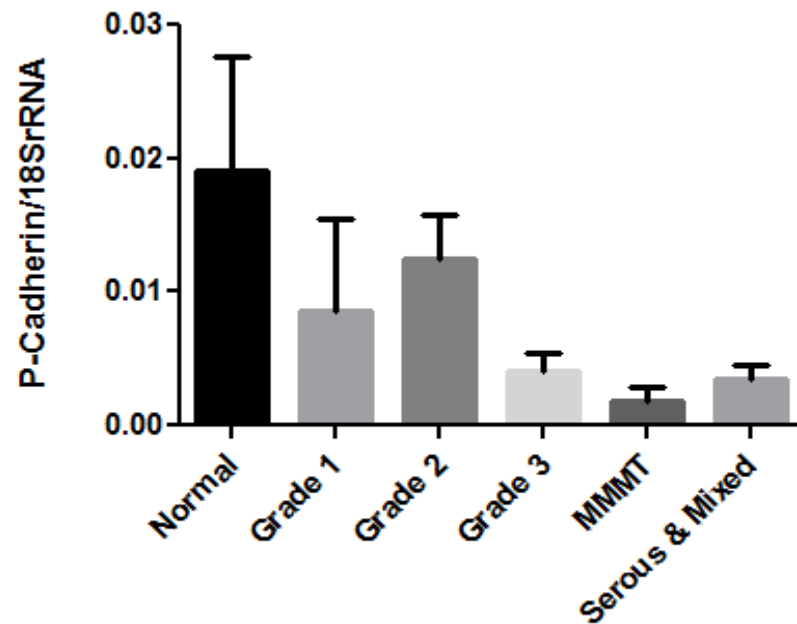


Figure 1. Expression of *P-cadherin* in normal endometrium and in endometrial carcinomas. This graph compares *P-cadherin* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of *P-cadherin* was decreased in carcinomas compared to normal, especially for the non-endometrioid carcinomas.

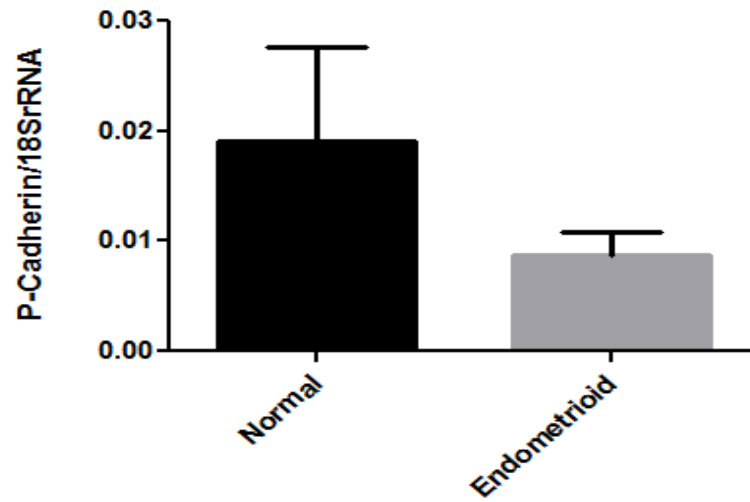


Figure 2. Expression of *P-cadherin* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is lower in the endometrioid tumors overall, but this is not statistically significant (p = 0.0882).

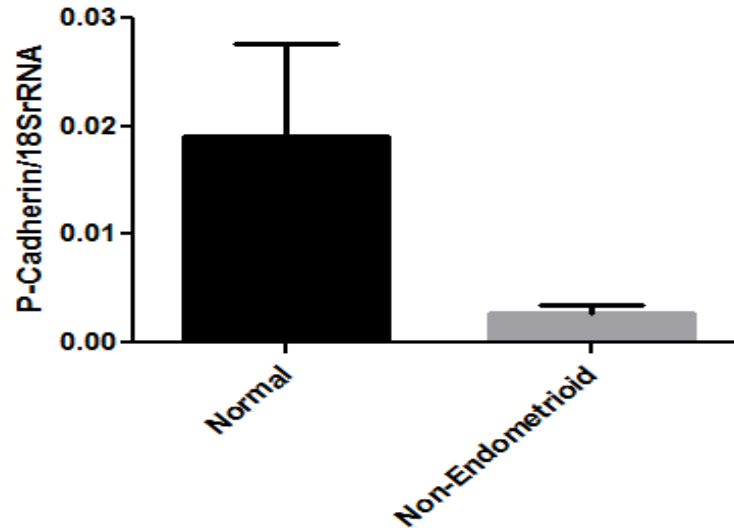


Figure 3. Expression of *P-cadherin* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0037).

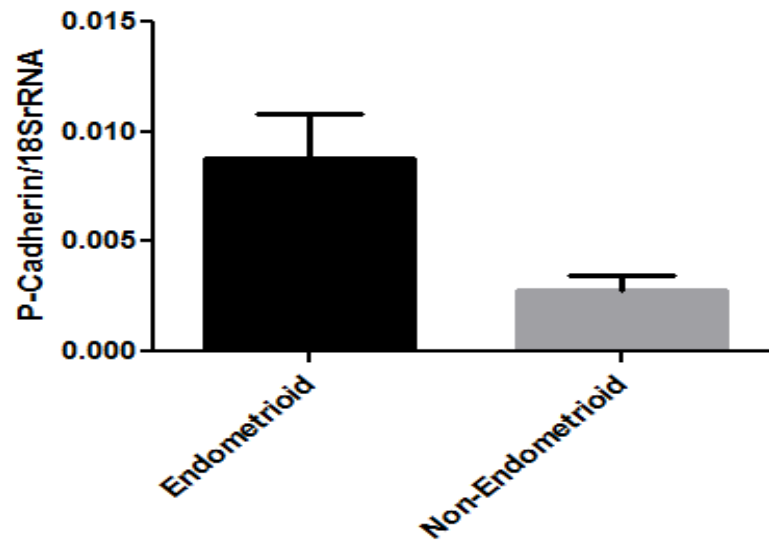


Figure 4. Expression of *P-cadherin* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression ($p = 0.0367$).

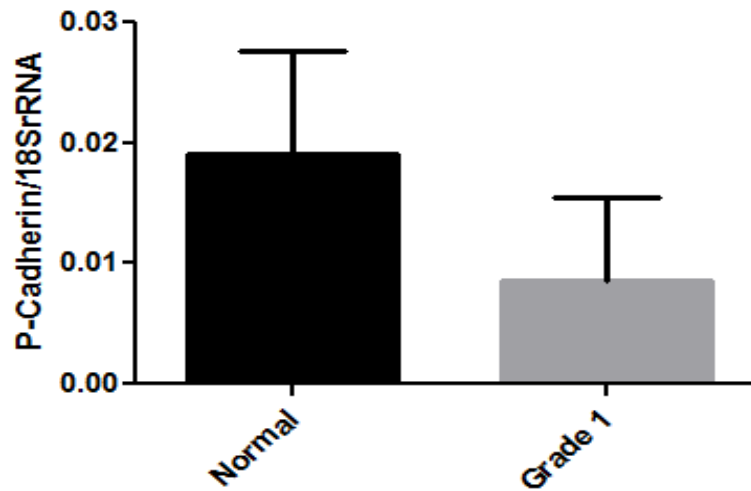


Figure 5. Expression of *P-cadherin* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Normal endometrium and grade 1 tumors have comparable expression ($p = 0.3616$).

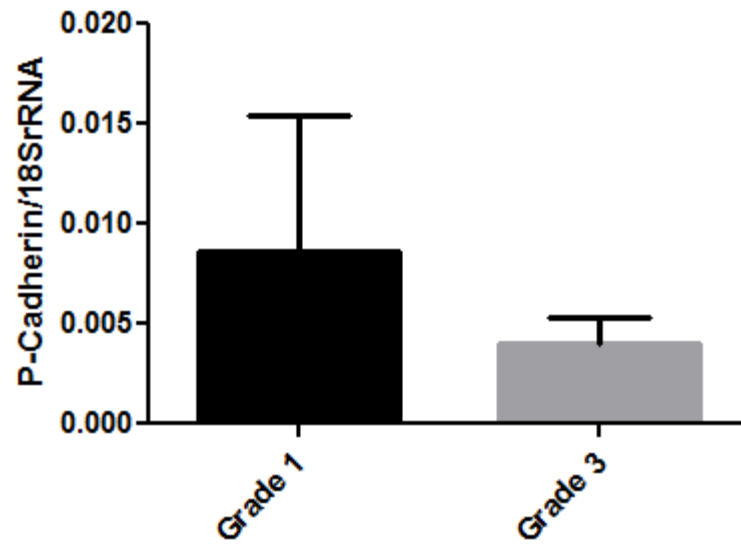


Figure 6. Expression of *P-cadherin* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). There is no significant difference between grade 1 and grade 3 ($p = 0.3963$).

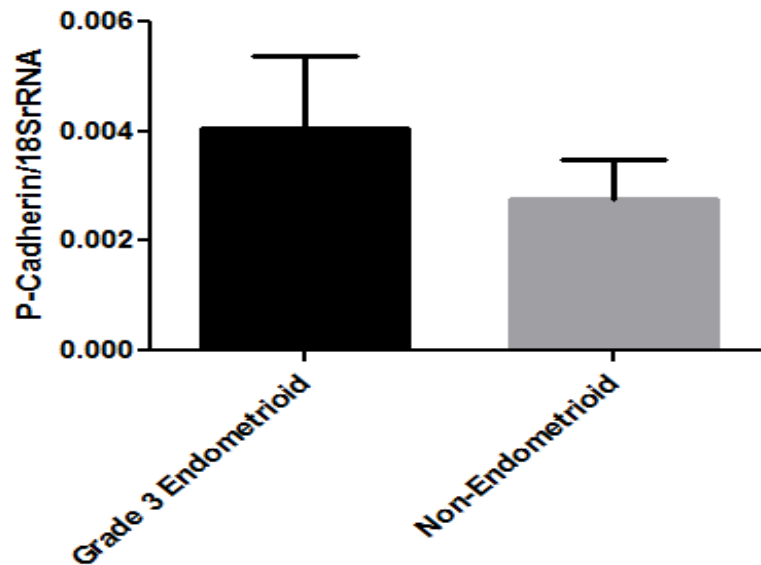


Figure 7. Expression of *P-cadherin* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significantly different ($p = 0.3516$).

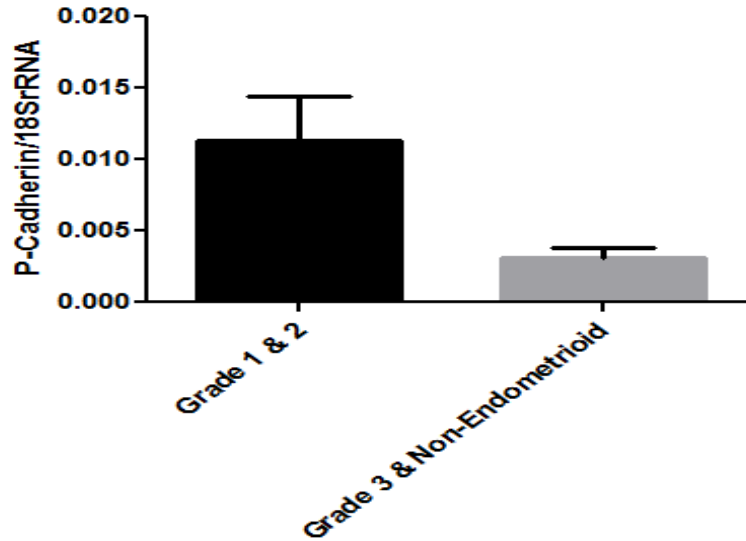


Figure 8. Expression of *P-cadherin* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0052).

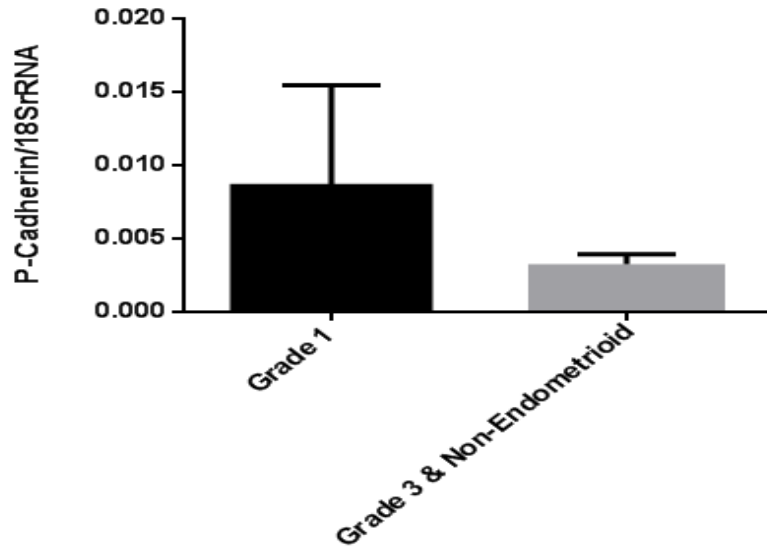


Figure 9. Expression of *P-cadherin* in grade 1 EECs (n = 9) and in grade 3 EECs and NEECs (n = 47). No significant difference in expression is observed between these groups (p = 0.1176).

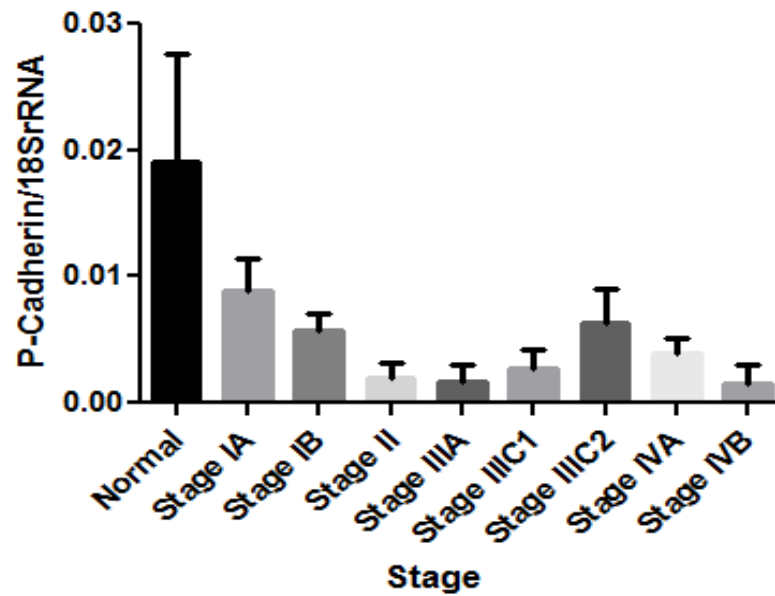


Figure 10. Expression of *P-cadherin* in normal endometrium and in endometrial carcinomas by FIGO stage.

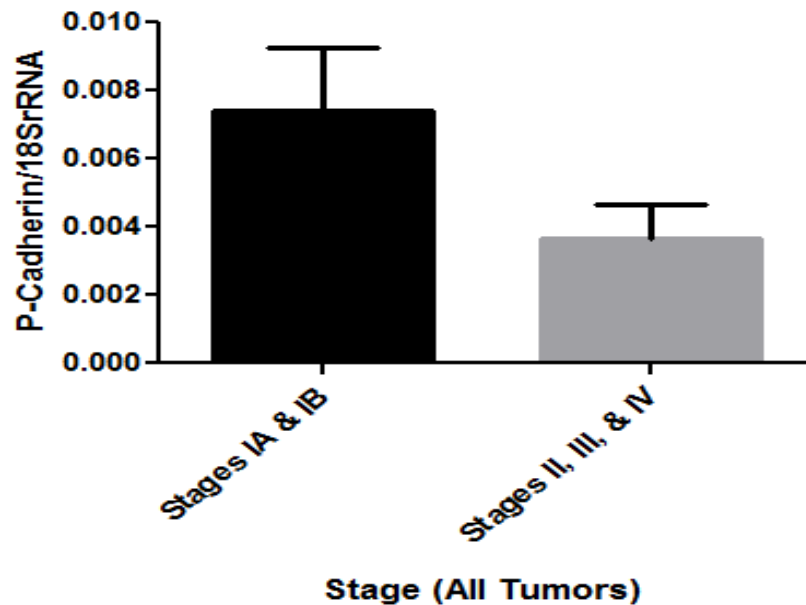


Figure 11. Expression of *P-cadherin* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.1575).

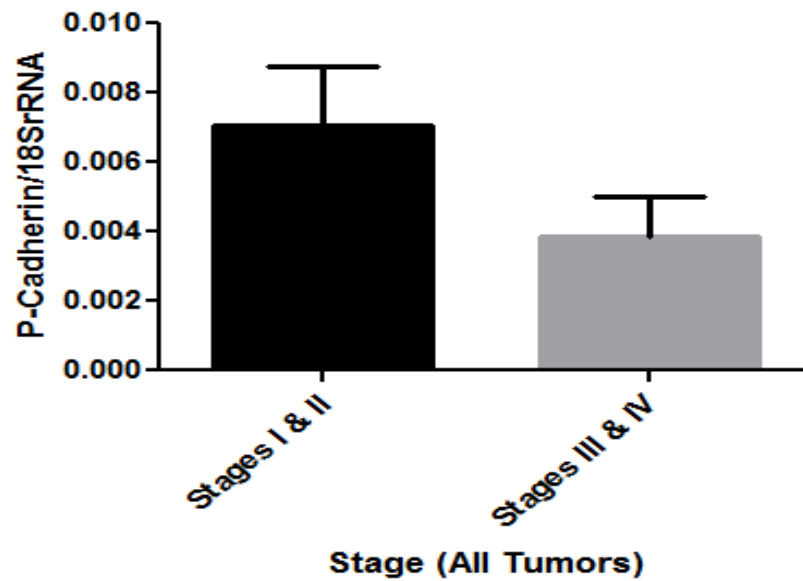


Figure 12. Expression of *P-cadherin* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant ($p = 0.2458$).

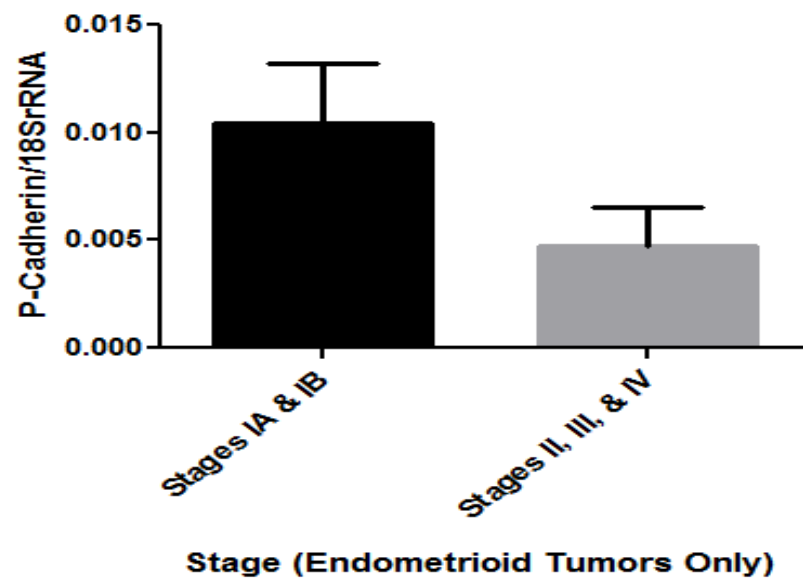


Figure 13. Expression of *P-cadherin* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.2128$).

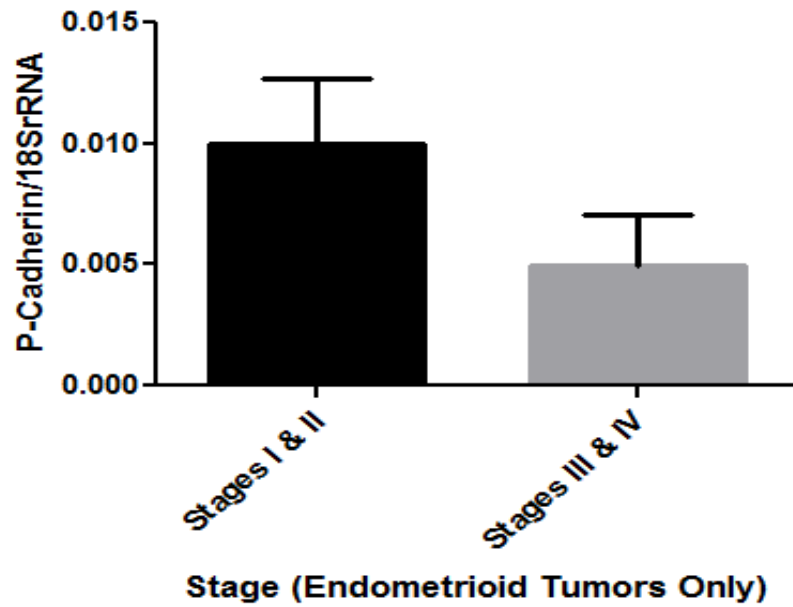


Figure 14. Expression of *P-cadherin* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.2985$).

Through immunohistochemistry, other groups have found higher *P-cadherin* expression in uterine clear cell carcinomas or serous carcinomas (10). High expression was also correlated with high FIGO grade, vascular invasion, and increasing FIGO stage (10). Our results are not in accordance with the literature. We found there to be significant decrease of *P-cadherin* expression in non-endometrioid endometrial carcinomas compared to normal endometrium ($p = 0.0037$), as well as between endometrioid endometrial carcinomas and non-endometrioid endometrioid carcinomas ($p = 0.0367$).

OLFM1

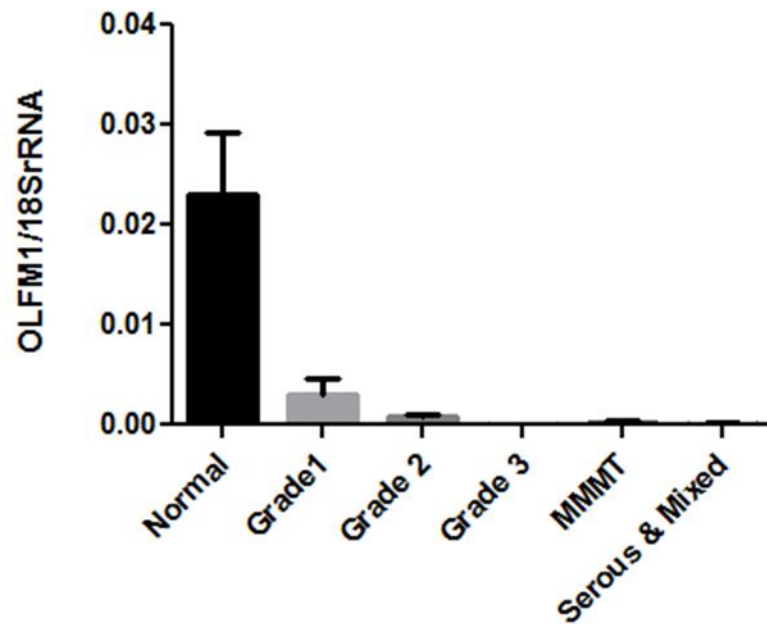


Figure 15. Expression of *OLFM1* in normal endometrium and in endometrial carcinomas. This graph compares *OLFM1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of *OLFM1* was significantly decreased in carcinomas compared to normal.

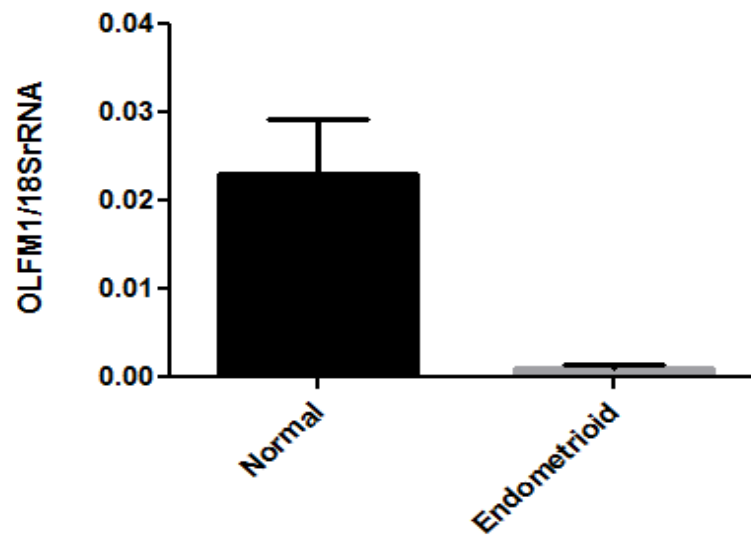


Figure 16. Expression of *OLFM1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

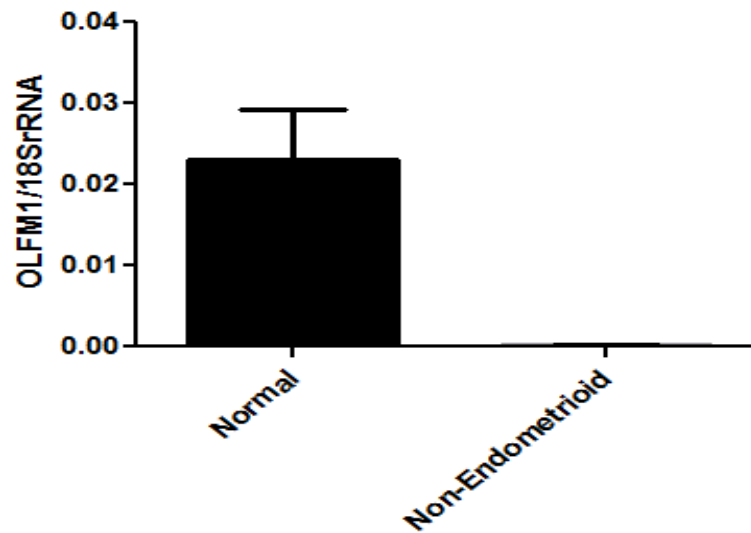


Figure 17. Expression of *OLFM1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

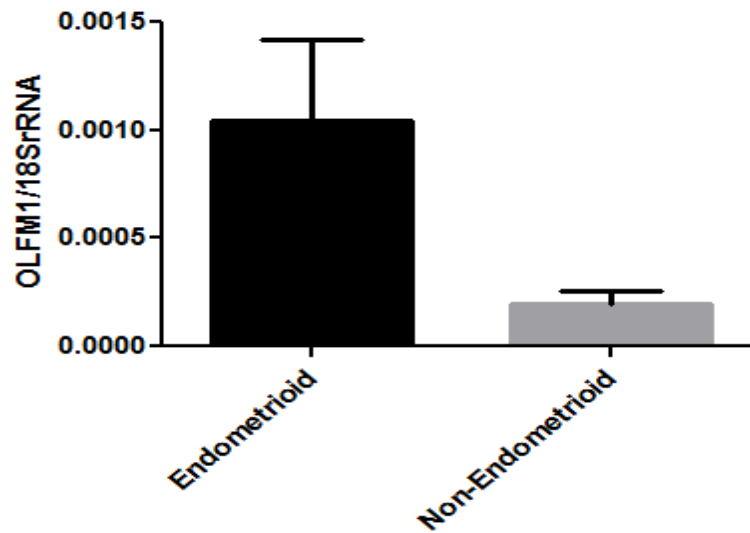


Figure 18. Expression of *OLFM1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Expression is lower in non-endometrioid tumors, but it is not statistically significantly (p = 0.0907).

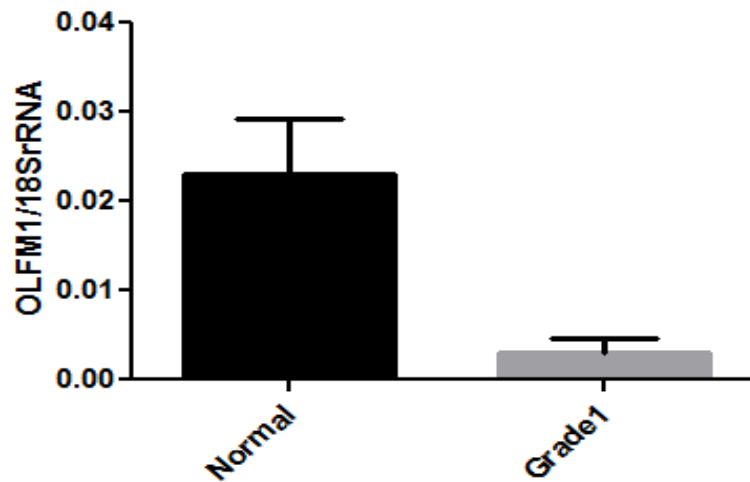


Figure 19. Expression of *OLFM1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Expression is significantly lower in grade 1 tumors (p = 0.0088).

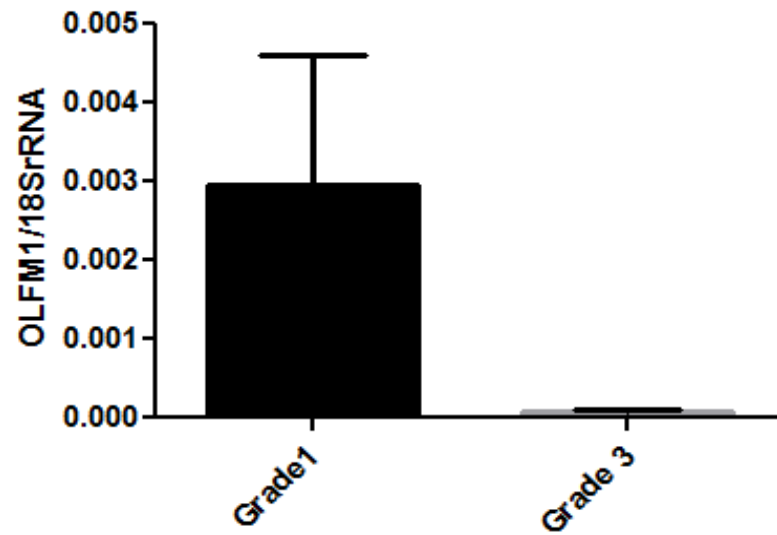


Figure 20. Expression of *OLFM1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Expression is significantly lower in grade 3 tumors (p = 0.0379).

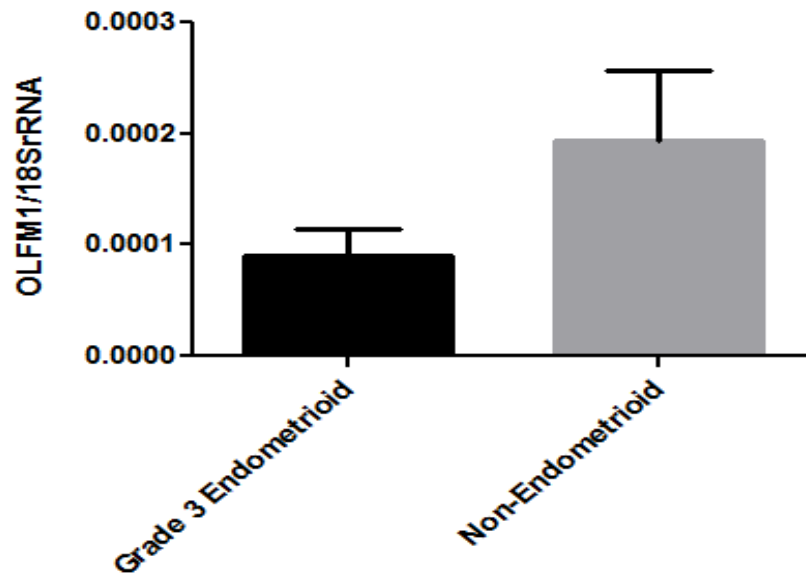


Figure 21. Expression of *OLFM1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significantly different (p = 0.2372).

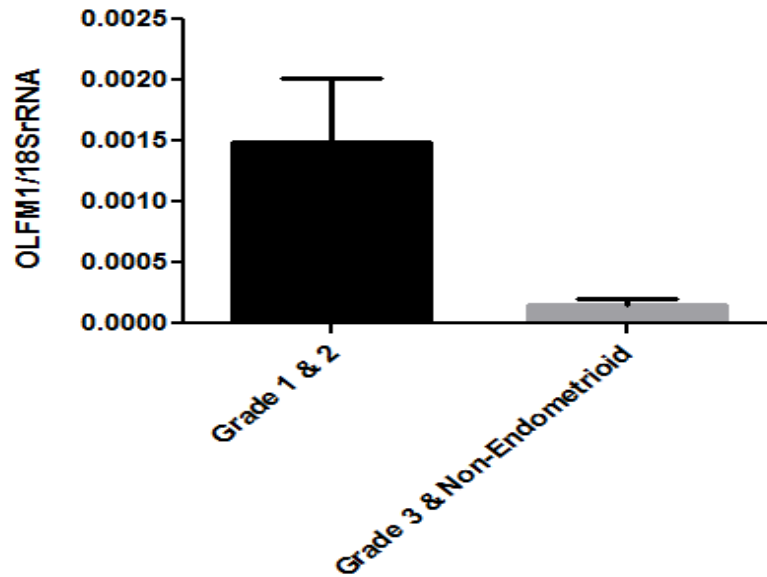


Figure 22. Expression of *OLFM1* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0053).

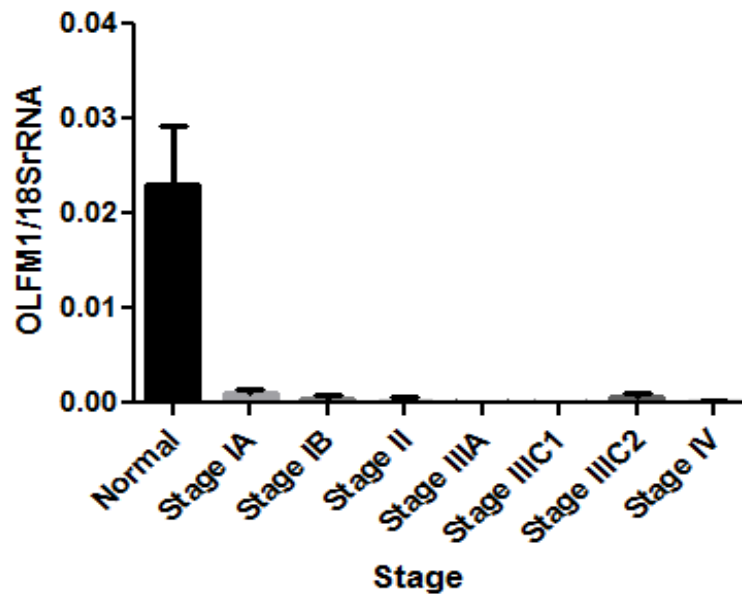


Figure 23. Expression of *OLFM1* in normal endometrium and in endometrial carcinomas by FIGO stage.

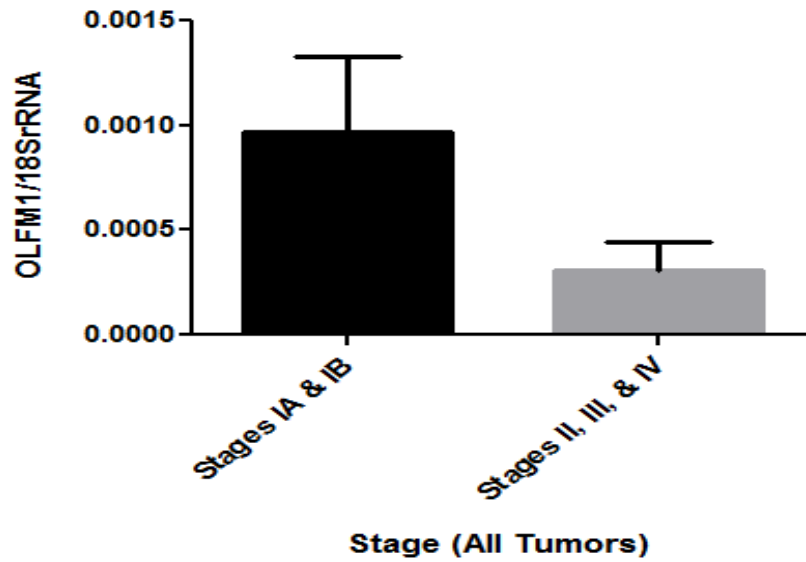


Figure 24. Expression of *OLFM1* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.1960).

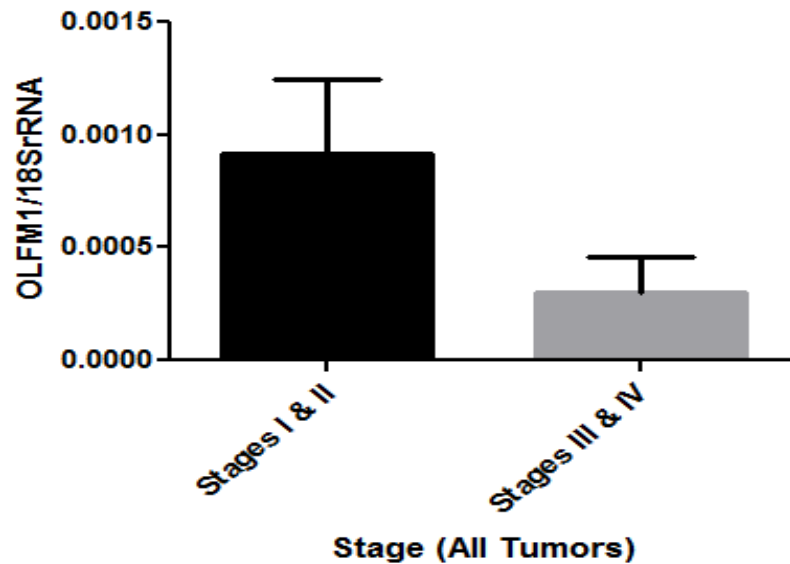


Figure 25. Expression of *OLFM1* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.2551).

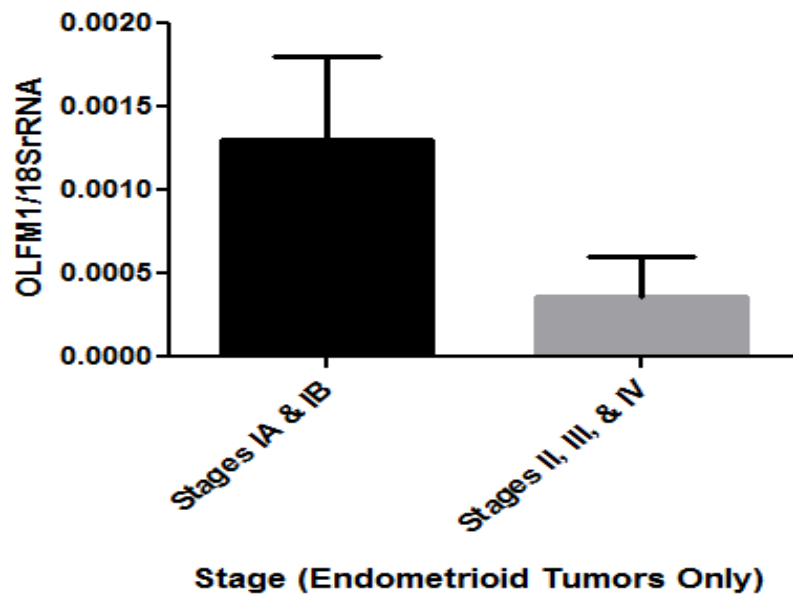


Figure 26. Expression of *OLFM1* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.2622$).

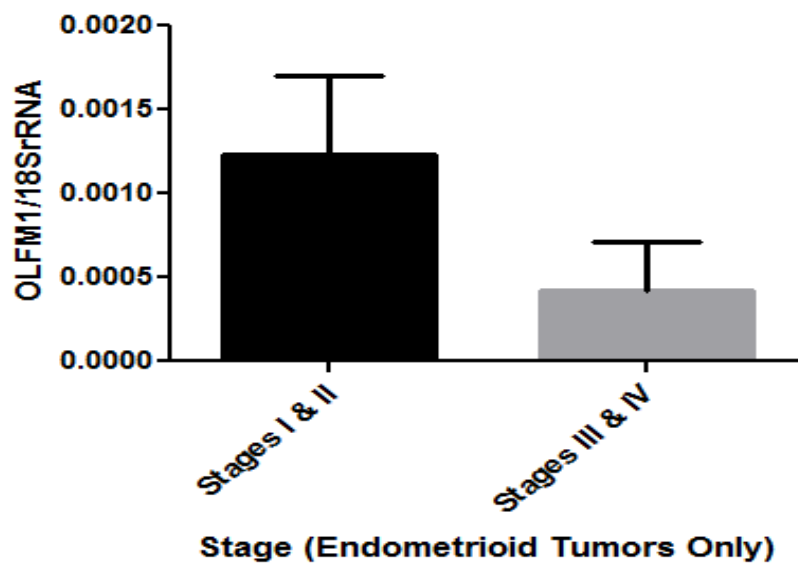


Figure 27. Expression of *OLFM1* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.3675$).

Based on our results, we found *OLFM1* expression to be significantly down-regulated in carcinomas, specifically in the higher grade endometrioid tumors and non-endometrioid tumors. These results are in accordance with the previously published data (12).

VAV3

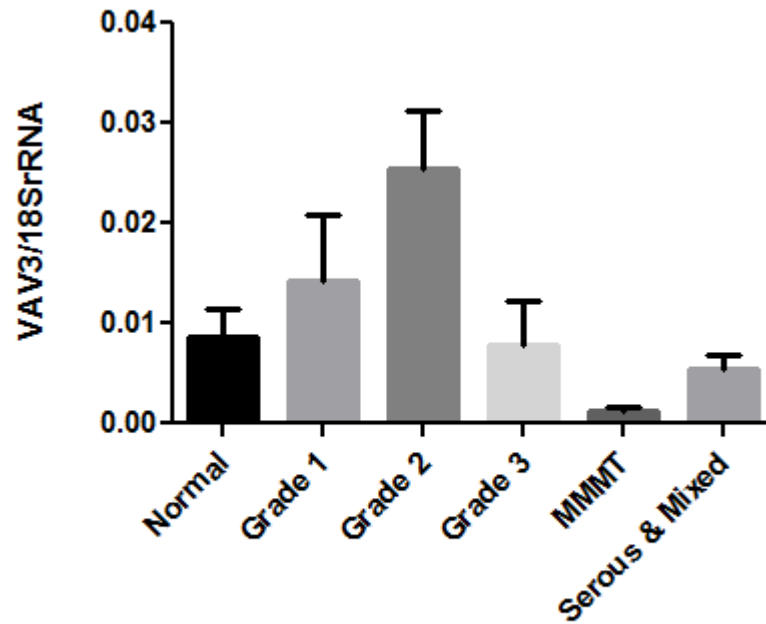


Figure 28. Expression of VAV3 in normal endometrium and in endometrial carcinomas. This graph compares VAV3 expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of VAV3 was increased in low grade endometrial carcinomas compared to normal, but significantly decreased for the non-endometrioid carcinomas.

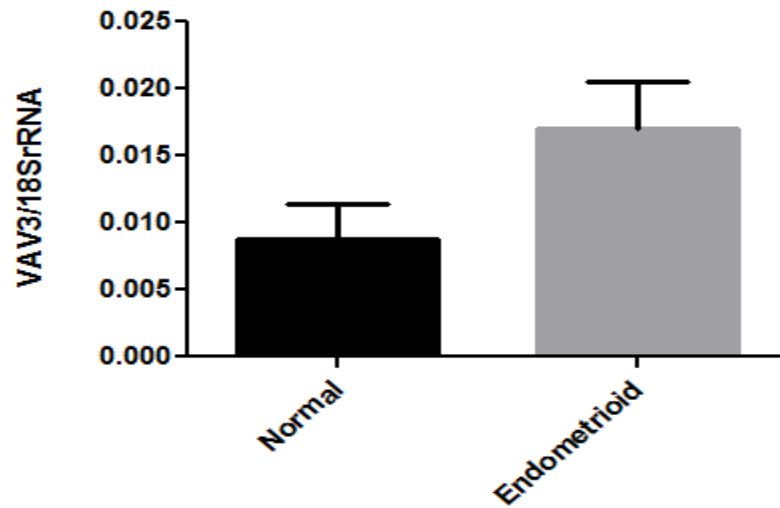


Figure 29. Expression of VAV3 in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is higher in the endometrioid tumors overall, but this difference is not statistically significant (p = 0.2822).

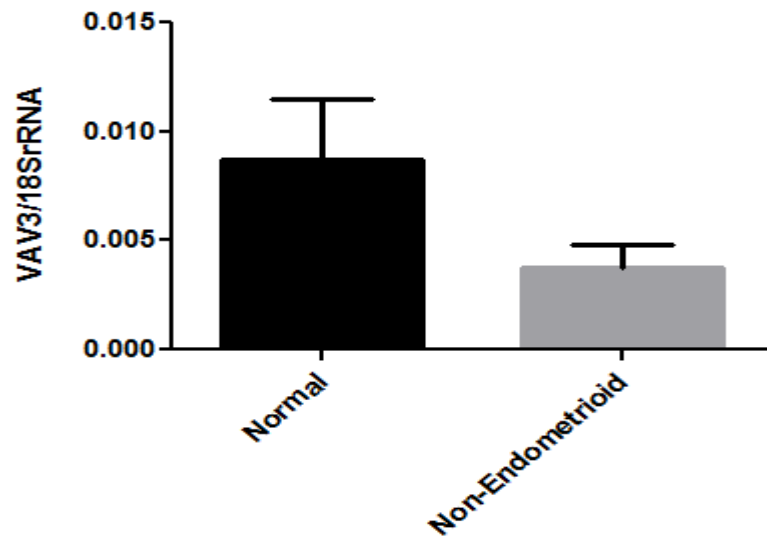


Figure 30. Expression of VAV3 in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.049).

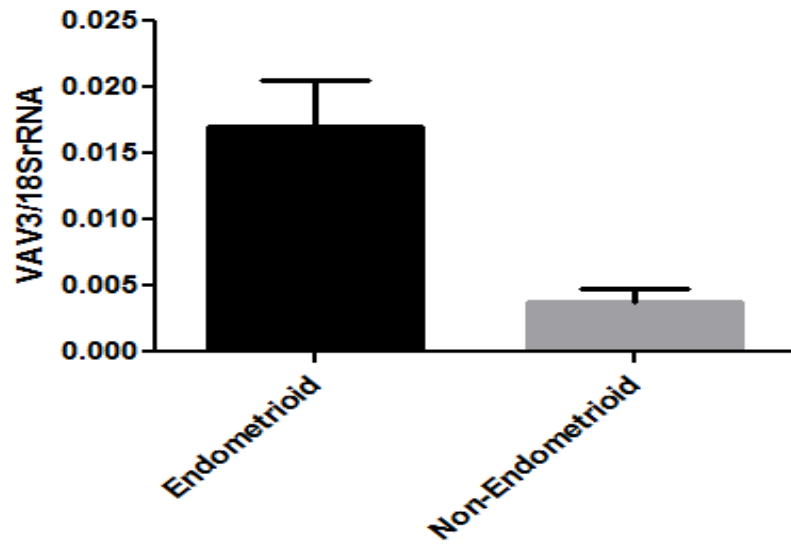


Figure 31. Expression of VAV3 in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0163).

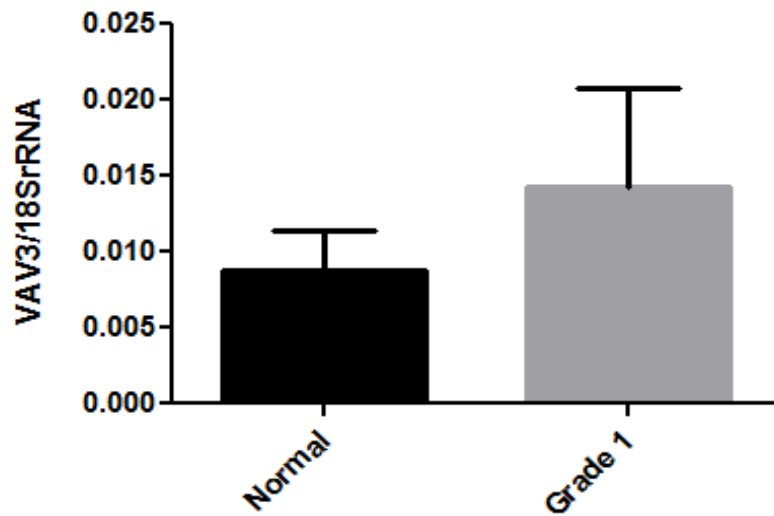


Figure 32. Expression of VAV3 in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Normal endometrium and grade 1 tumors have comparable expression (p = 0.4313).

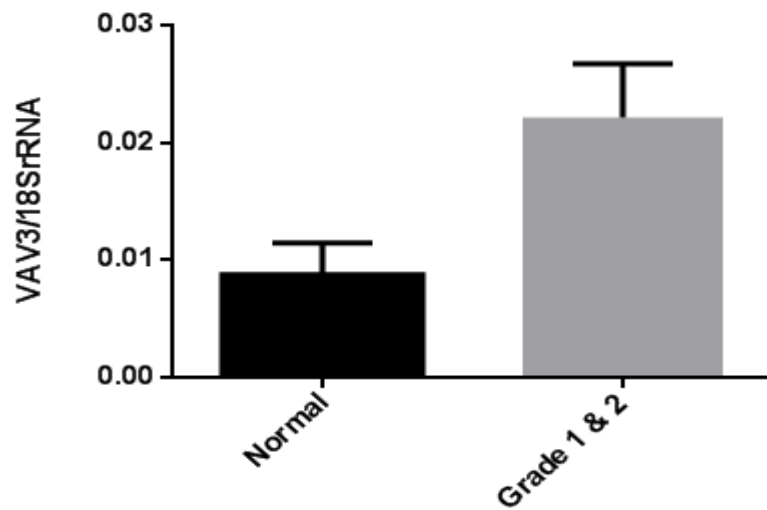


Figure 33. Expression of VAV3 in normal endometrium (n = 10) and grade 1 and 2 endometrioid endometrial carcinomas (n = 31). Normal endometrium and grades 1 and 2 tumors have comparable expression (p = 0.1134).

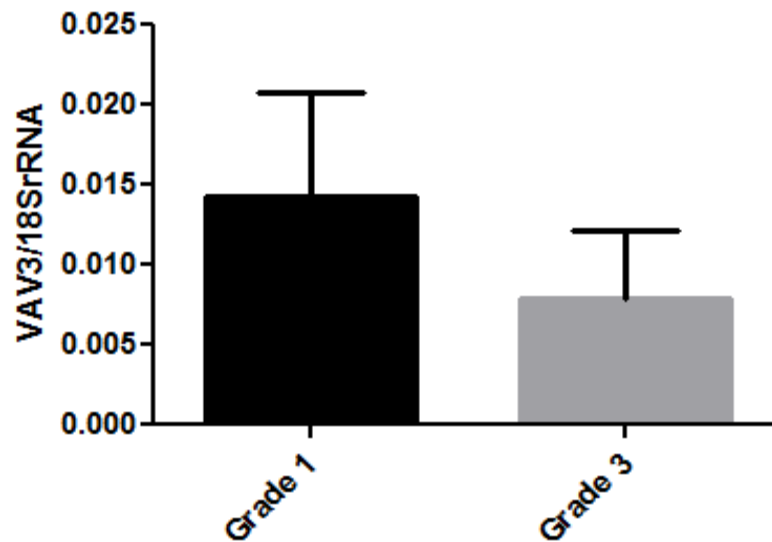


Figure 34. Expression of VAV3 in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). There is no significant difference in expression between grade 1 and grade 3 (p = 0.4076).

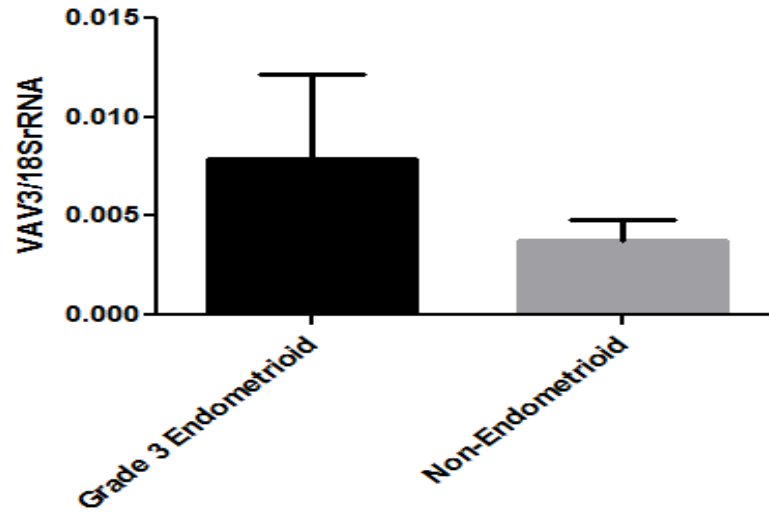


Figure 35. . Expression of VAV3 in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significant ($p = 0.3237$).

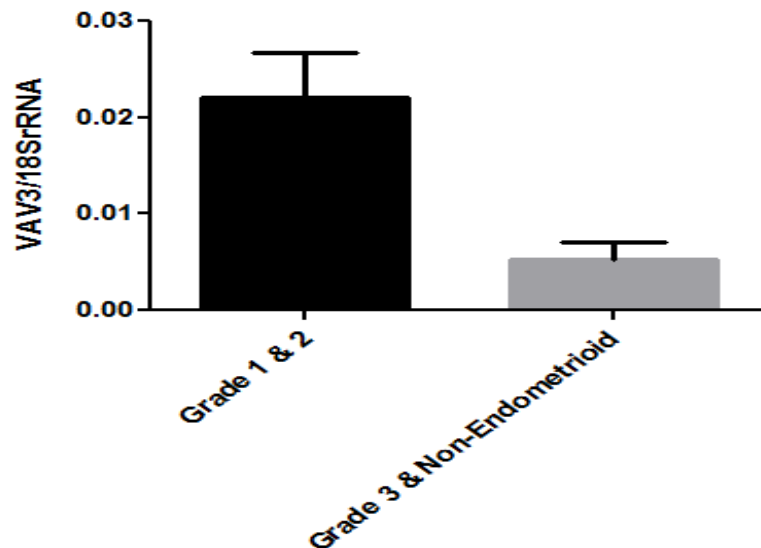


Figure 36. Expression of VAV3 in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group ($p = 0.0003$).

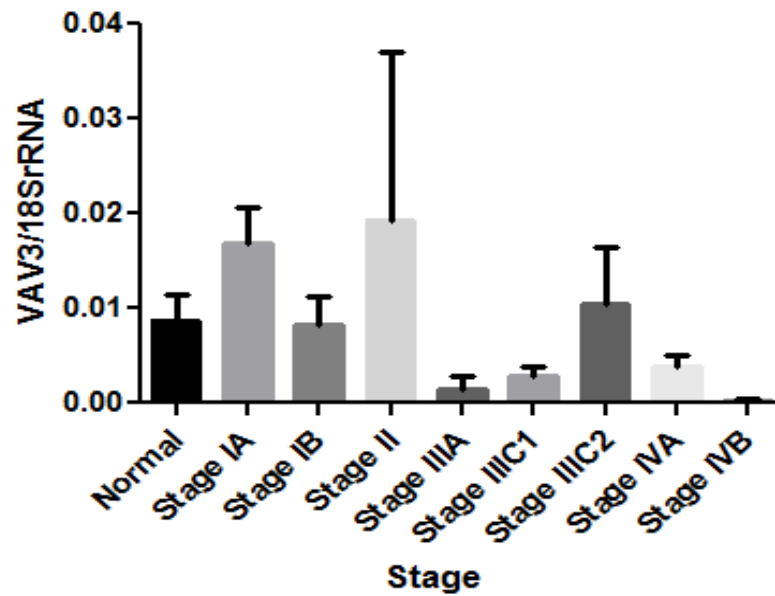


Figure 37. Expression of VAV3 in normal endometrium and in endometrial carcinomas by FIGO stage.

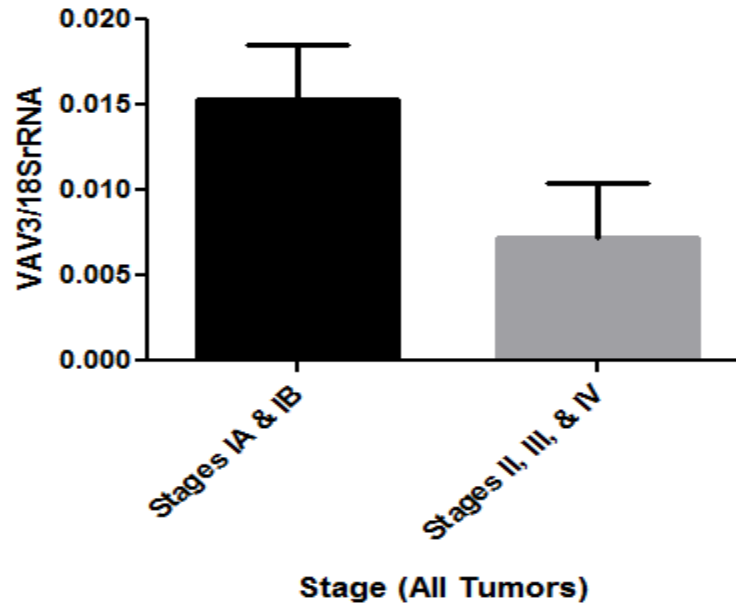


Figure 38. Expression of VAV3 in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.1000).

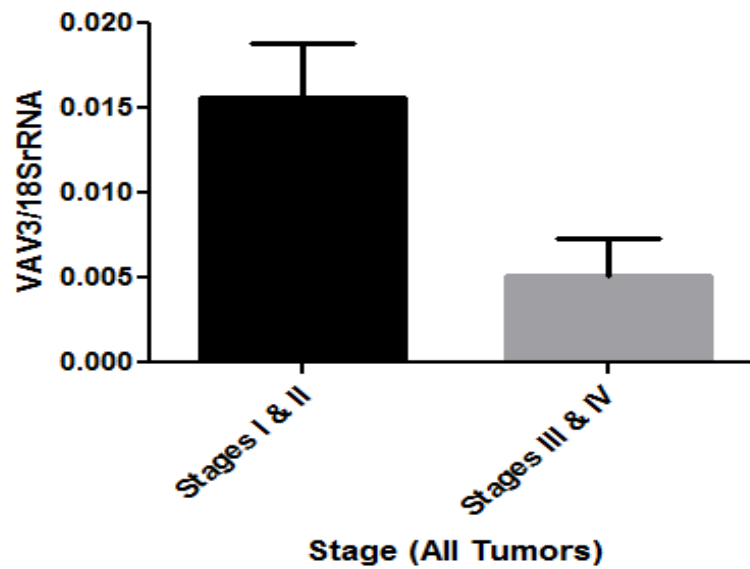


Figure 39. Expression of VAV3 in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have significantly lower expression ($p = 0.0389$).

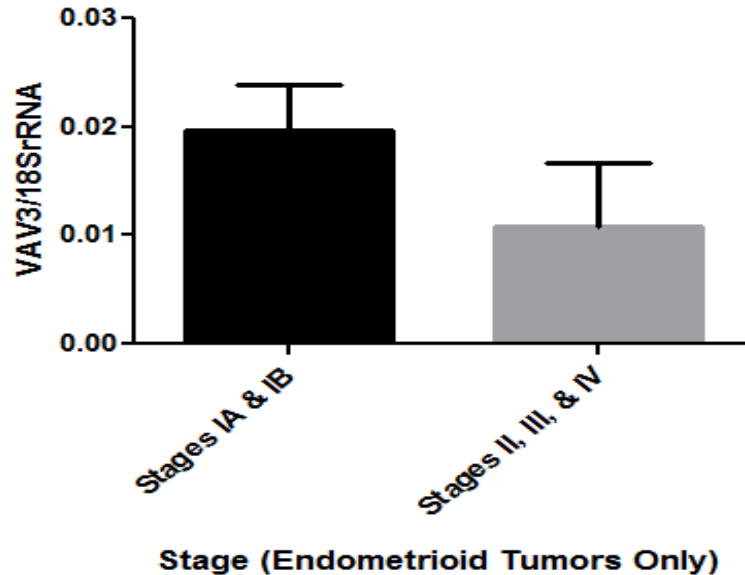


Figure 40. Expression of VAV3 in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.2443$).

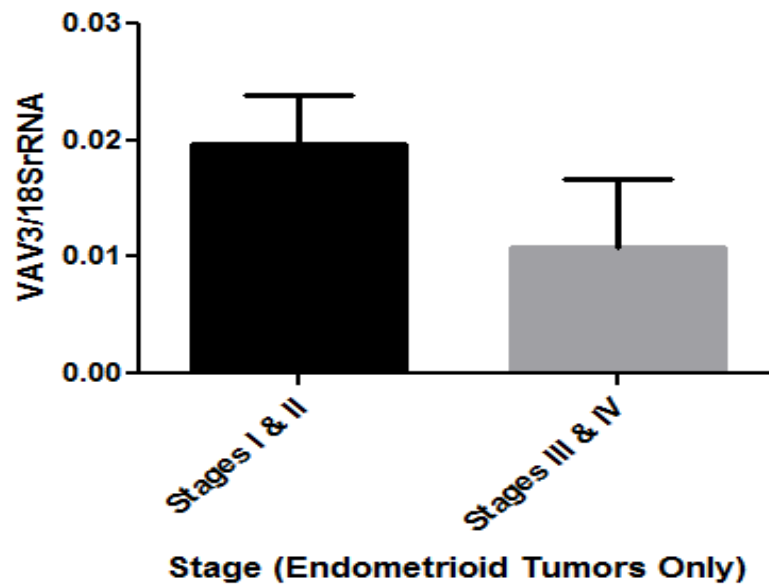


Figure 41. Expression of VAV3 in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.2443$).

Our data showed VAV3 to be significantly down-regulated in non-endometrioid carcinomas compared to endometrioid carcinomas. Literature has shown VAV3 to be overexpressed in correlation with poorly differentiated breast carcinomas through immunohistochemistry and western blot analysis (13). However, our results show a higher expression in well differentiated endometrial carcinomas.

SPRY1

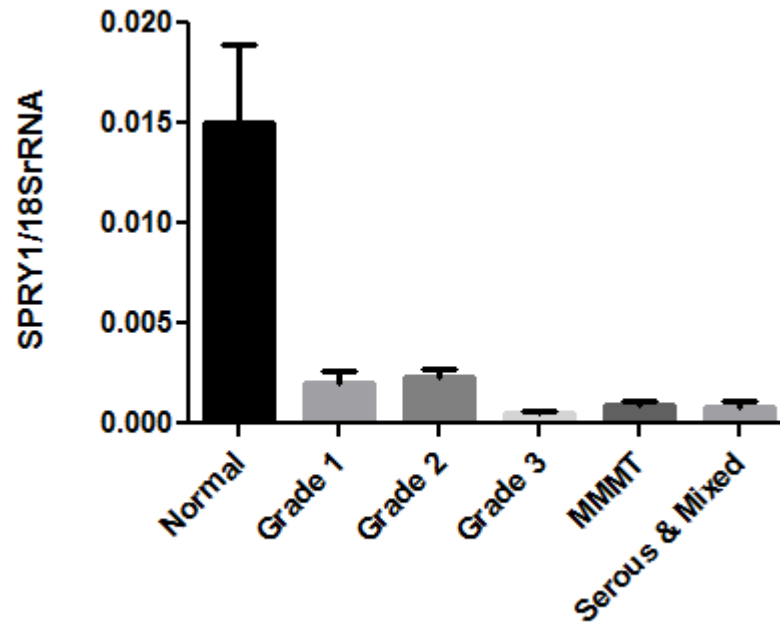


Figure 42. Expression of *SPRY1* in normal endometrium and in endometrial carcinomas. This graph compares *SPRY1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). *SPRY1* expression was significantly decreased in endometrial carcinomas compared to normal.

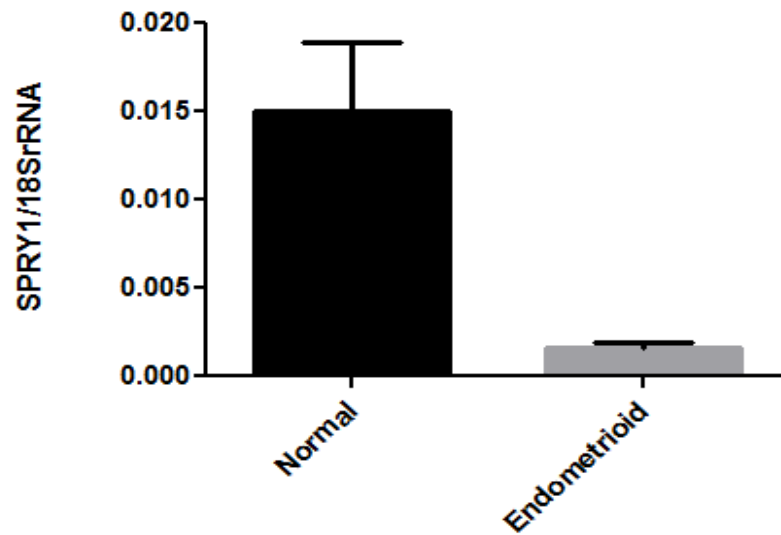


Figure 43. Expression of *SPRY1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

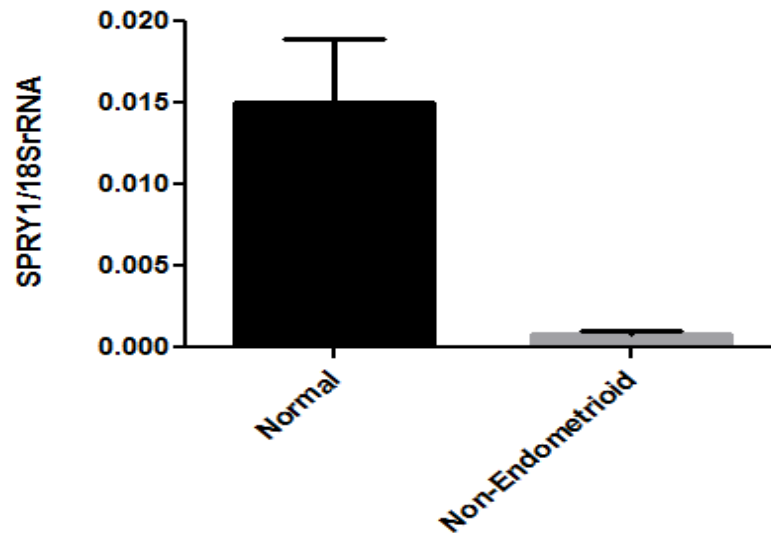


Figure 44. Expression of *SPRY1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

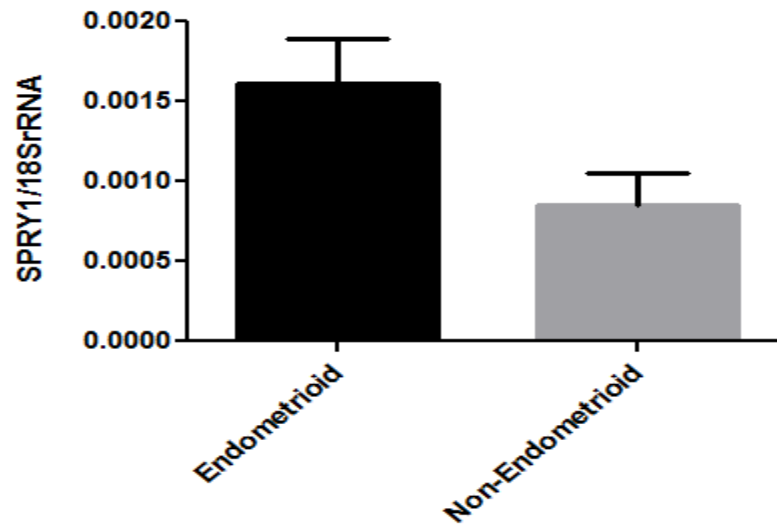


Figure 45. Expression of *SPRY1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Endometrioid tumors and non-endometrioid tumors have comparable expression (p = 0.0666).

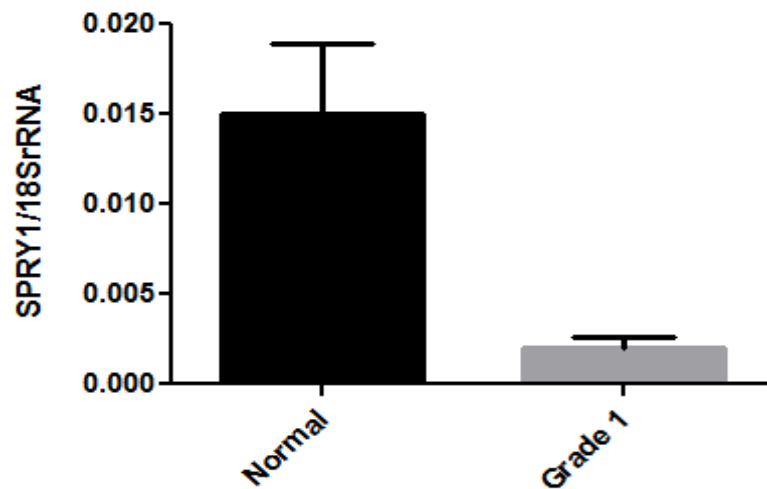


Figure 46. Expression of *SPRY1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Grade 1 tumors have significantly lower expression (p = 0.0055).

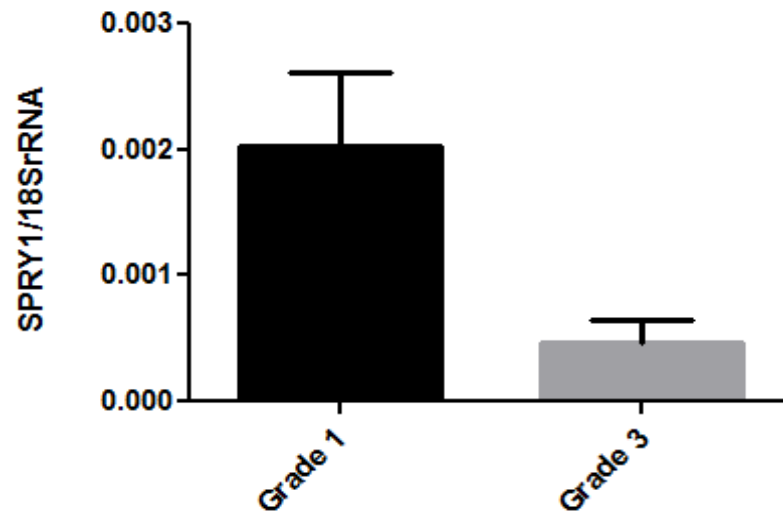


Figure 47. Expression of *SPRY1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0049).

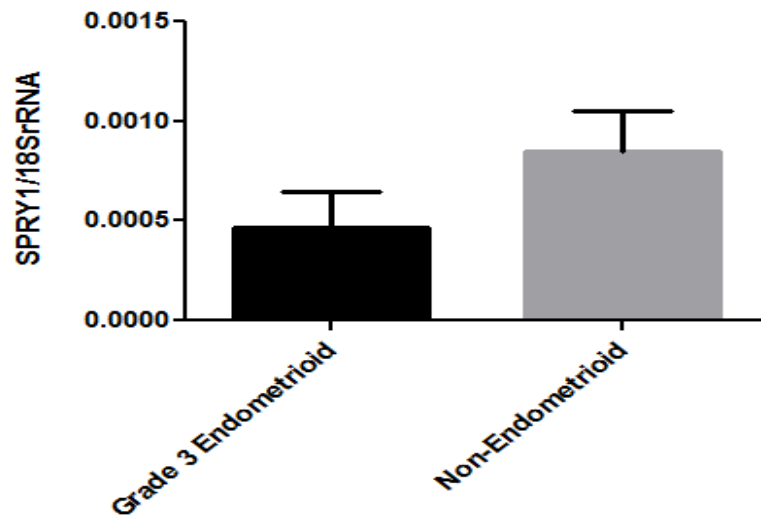


Figure 48. Expression of *SPRY1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significantly different (p = 0.1993).

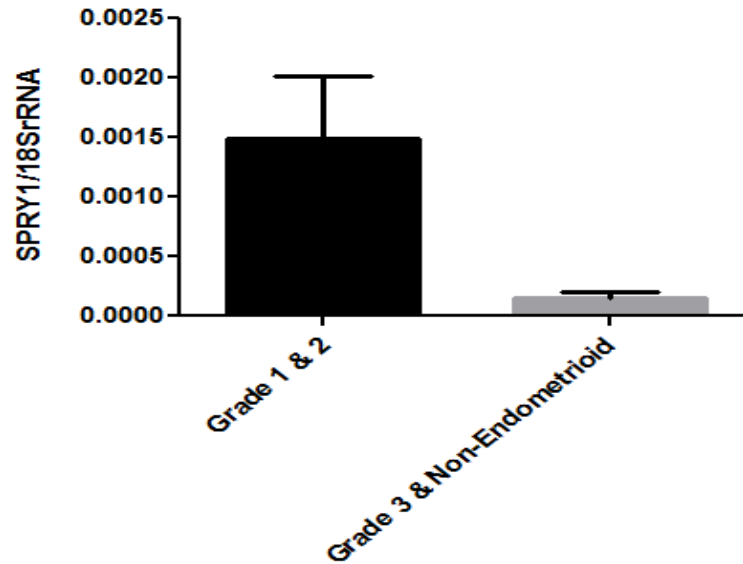


Figure 49. Expression of *SPRY1* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0053).

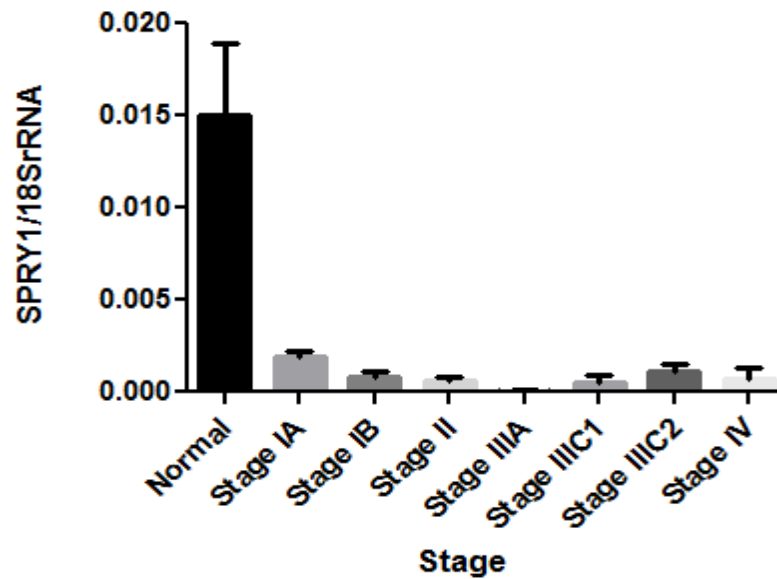


Figure 50. Expression of *SPRY1* in normal endometrium and in endometrial carcinomas by FIGO stage.

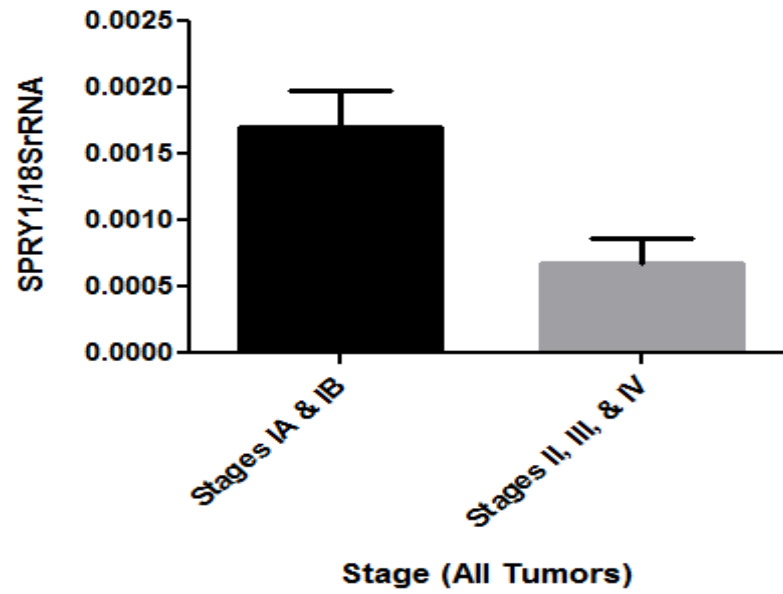


Figure 51. Expression of *SPRY1* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression (p = 0.0126).

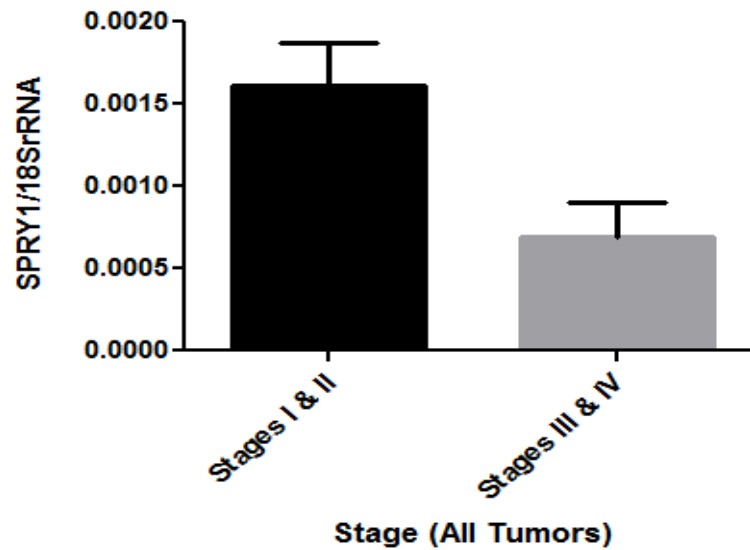


Figure 52. Expression of *SPRY1* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have significantly lower expression (p = 0.0322).

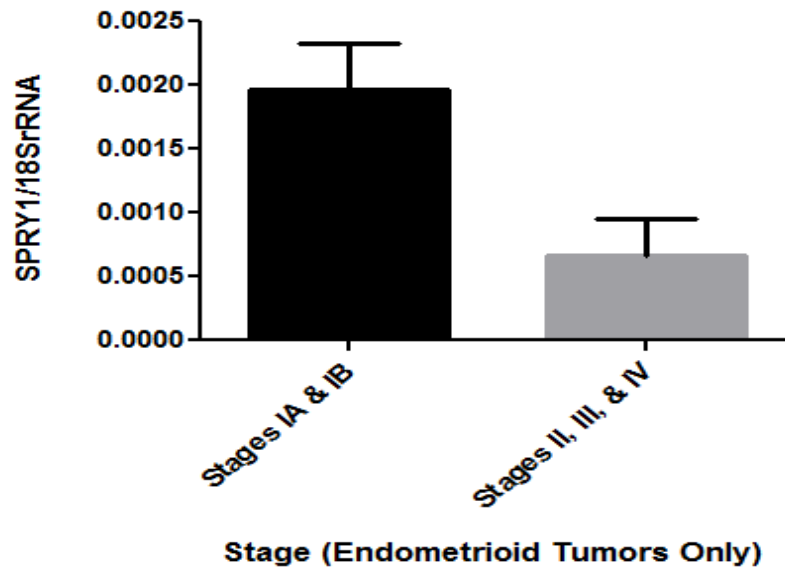


Figure 53. Expression of *SPRY1* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. Higher stages have significantly lower expression ($p = 0.0371$).

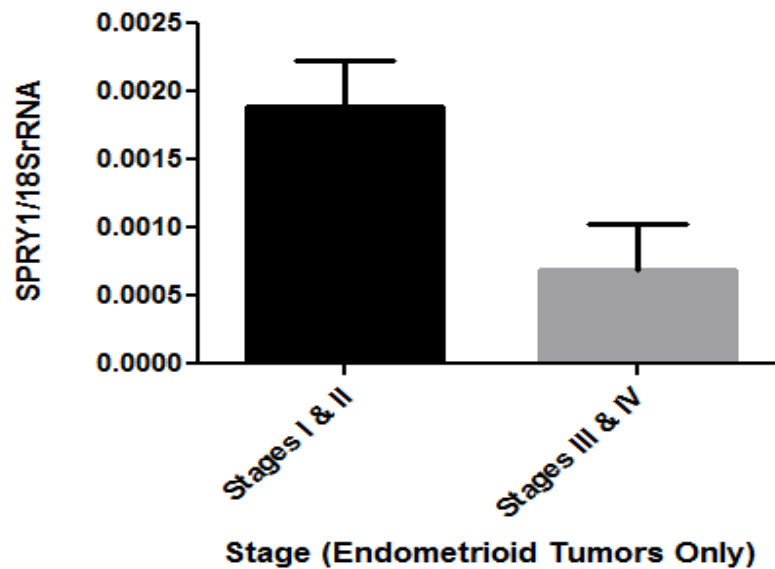


Figure 54. Expression of *SPRY1* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.0704$).

Our results found *SPRY1* expression to be significantly different when comparing several endometrial tumor subtypes. *SPRY1* expression is significantly down-regulated in correlation with increasing FIGO grade ($p = <0.0001$). *SPRY1* expression is significantly lower in grade 1 endometrioid endometrial carcinomas compared to normal endometrium. While *SPRY1* expression level have not been previously studied in endometrial carcinomas, this gene has also been found to be down-regulated in other cancers, such as prostate and breast (15, 16).

PIK3R1

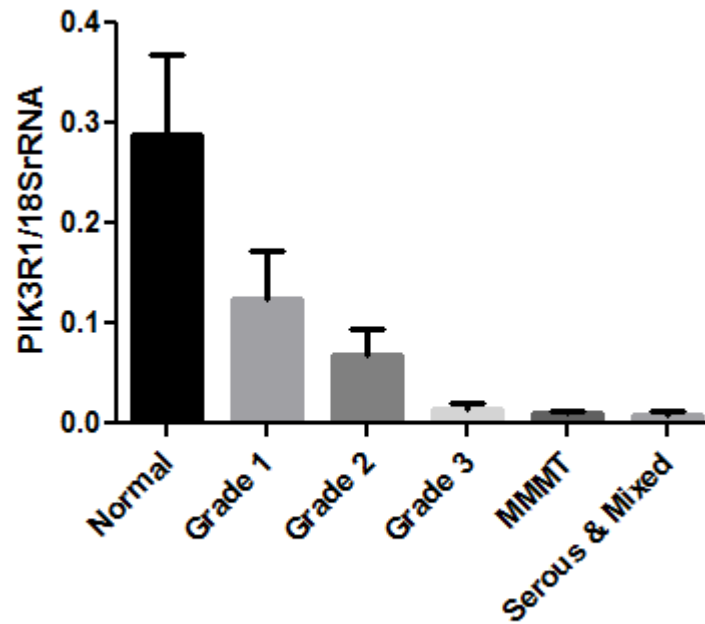


Figure 55. Expression of *PIK3R1* in normal endometrium and in endometrial carcinomas. This graph compares *PIK3R1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression is significantly lower in endometrial carcinomas compared to normal.

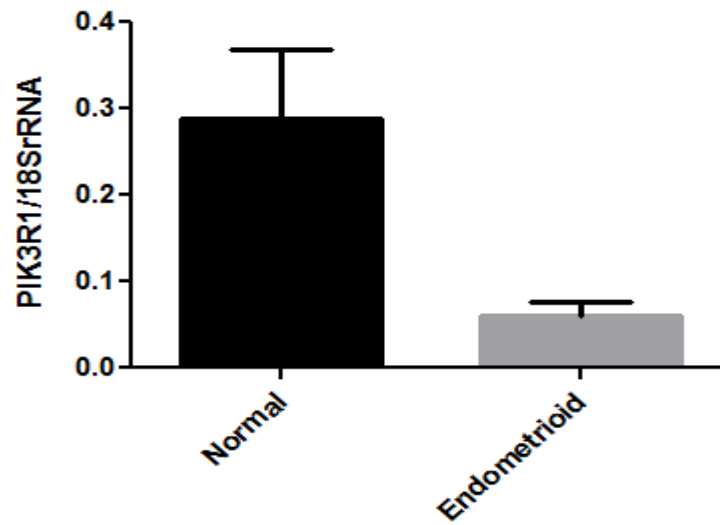


Figure 56. Expression of *PIK3R1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

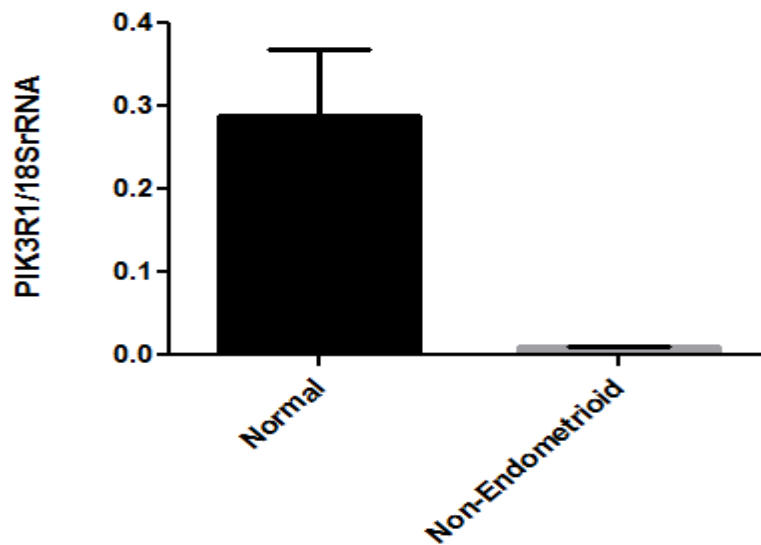


Figure 57. Expression of *PIK3R1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

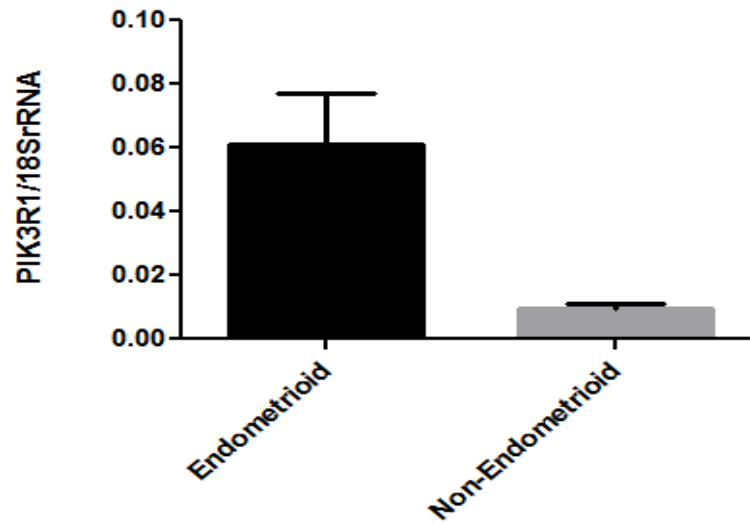


Figure 58. Expression of *PIK3R1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0200).

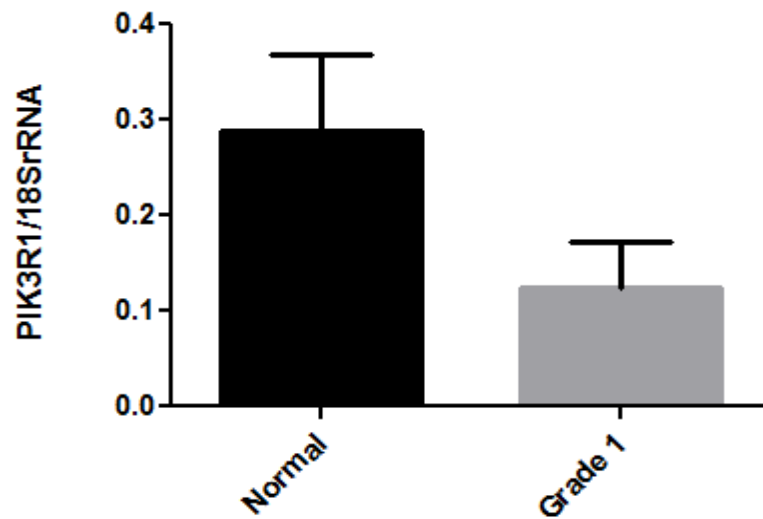


Figure 59. Expression of *PIK3R1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Normal endometrium and grade 1 tumors have comparable expression (p = 0.1073).

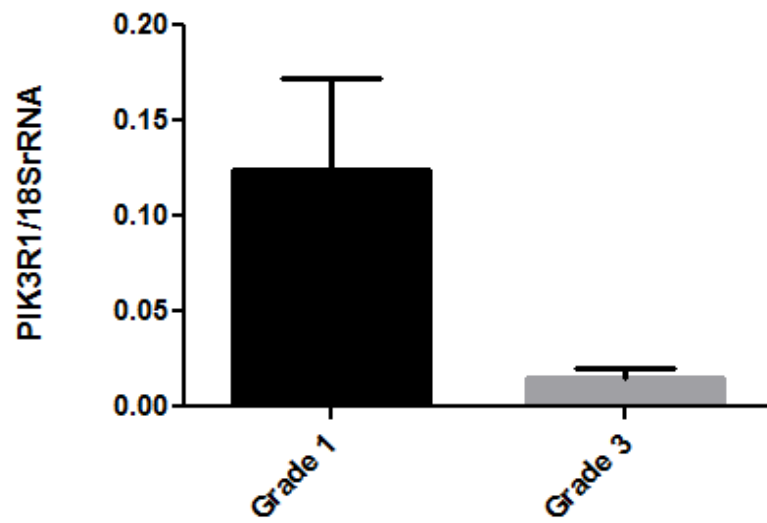


Figure 60. . Expression of *PIK3R1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0057).

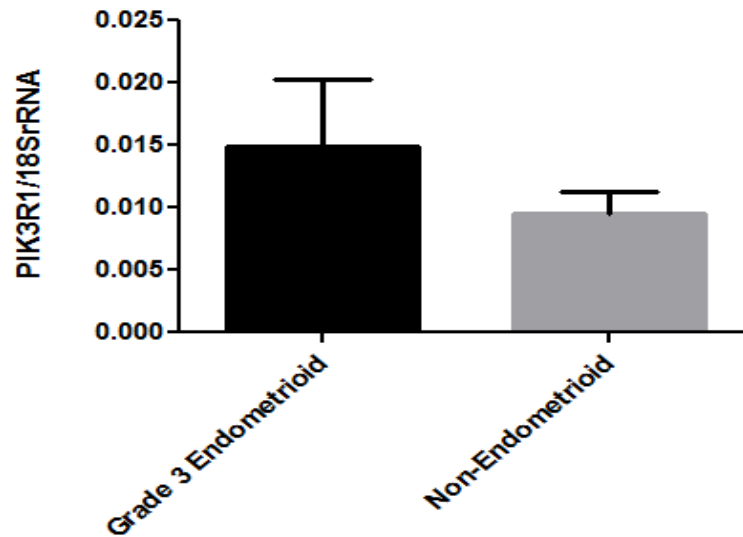


Figure 61. Expression of *PIK3R1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significantly different (p = 0.2599).

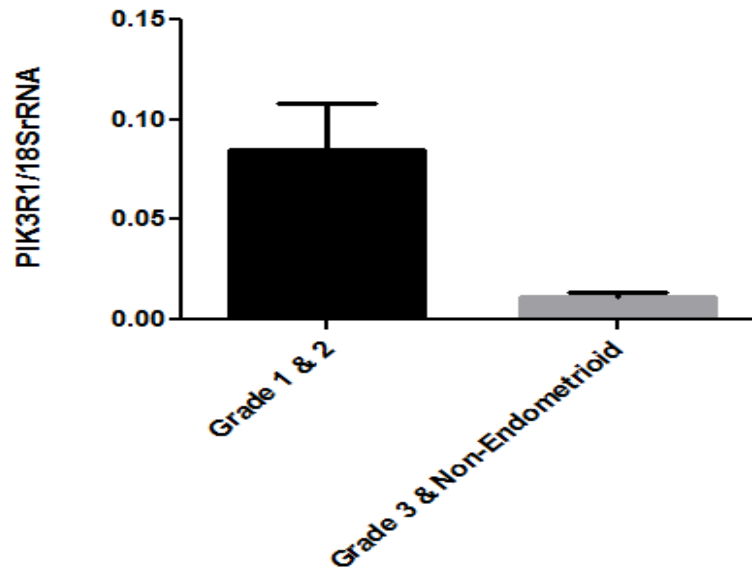


Figure 62. Expression of *PIK3R1* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0005).

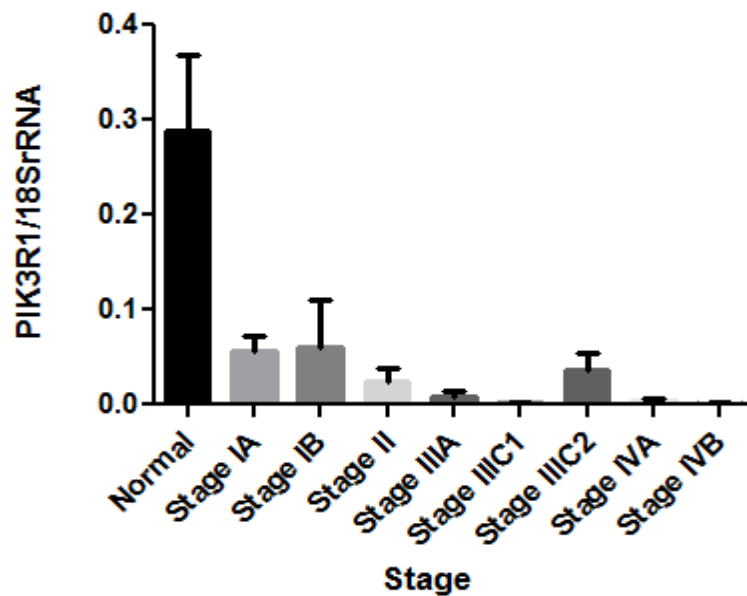


Figure 63. Expression of *PIK3R1* in normal endometrium and in endometrial carcinomas by FIGO stage.

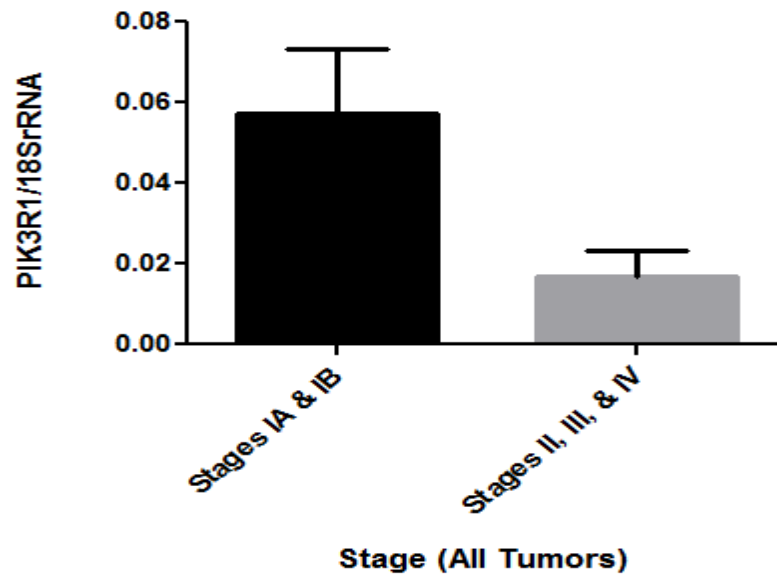


Figure 64. Expression of *PIK3R1* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.0692).

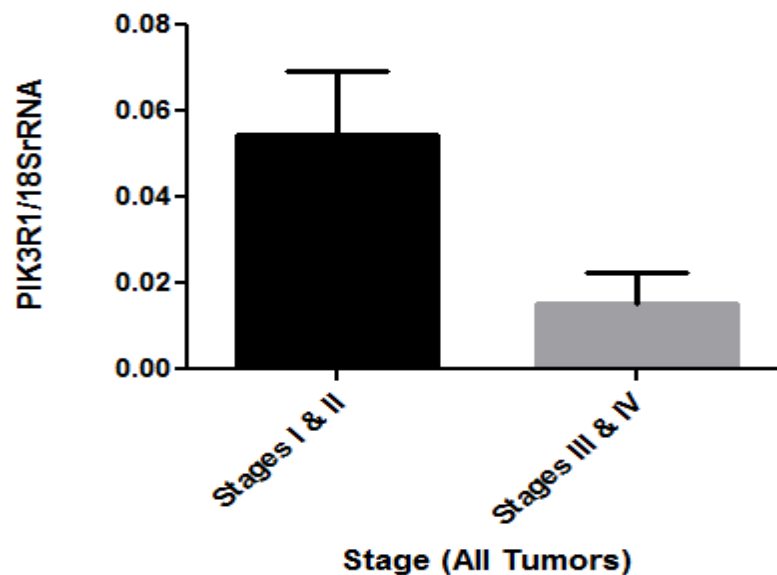


Figure 65. Expression of *PIK3R1* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.0936).

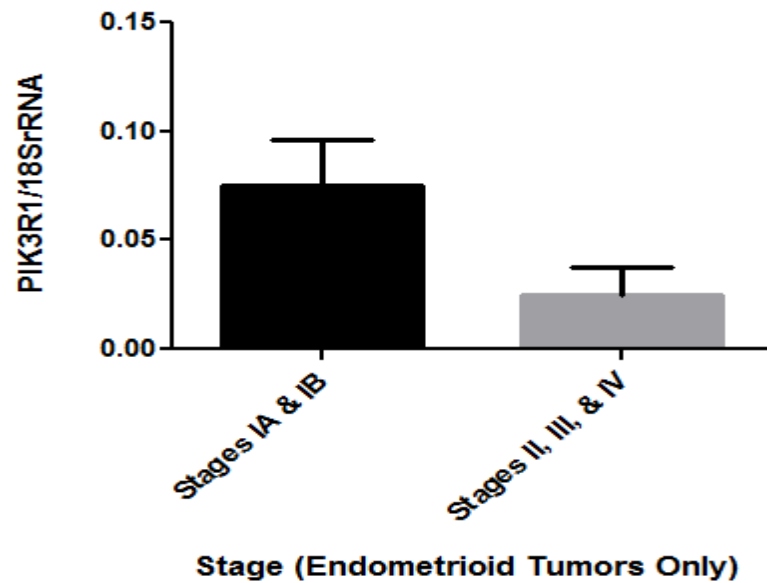


Figure 66. Expression of *PIK3R1* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.1650$).

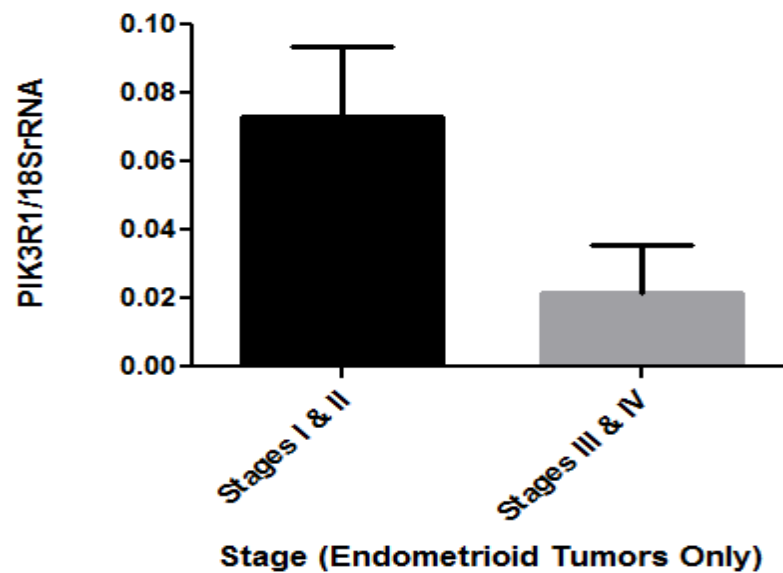


Figure 67. Expression of *PIK3R1* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.1755$).

We found *PIK3R1* gene expression to be significantly down-regulated in non-endometrioid carcinomas compared to endometrioid carcinomas. While advanced stage tumors tend to have lower expression, these differences are not statistically significant when comparing to early stages.

PEG3

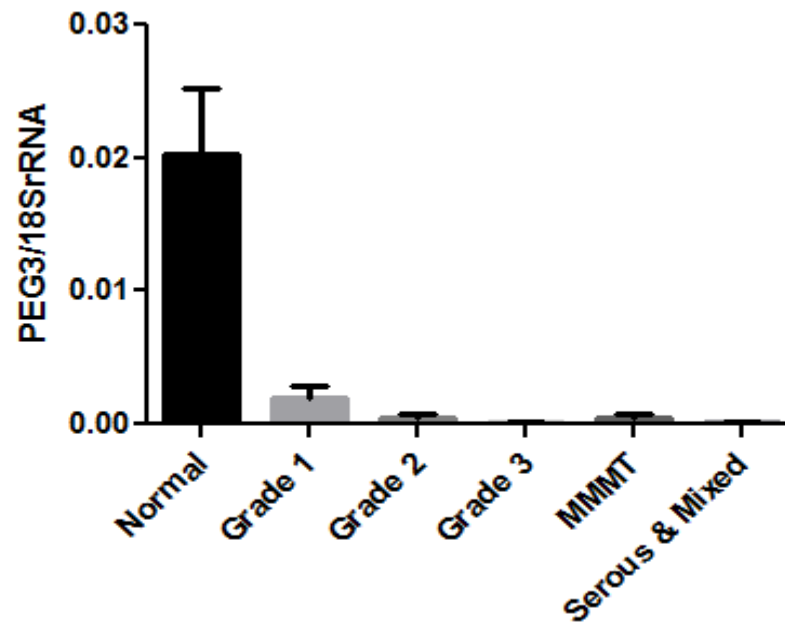


Figure 68. Expression of *PEG3* in normal endometrium and in endometrial carcinomas. This graph compares *PEG3* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression was significantly decreased in endometrial carcinomas compared to normal.

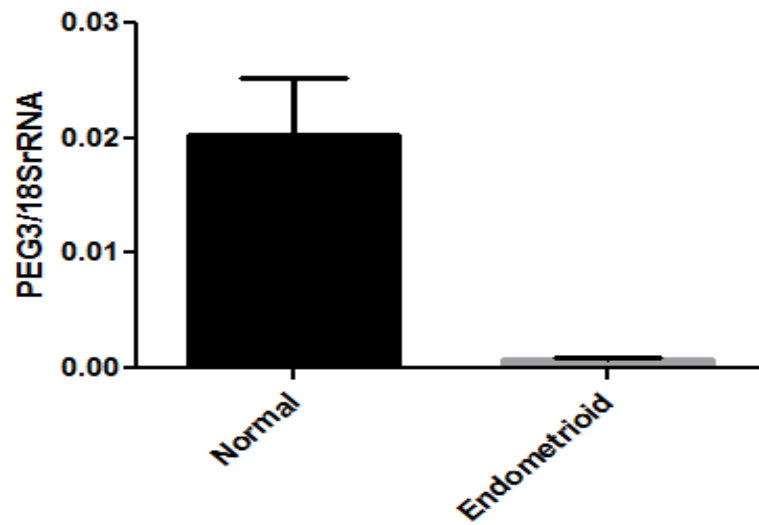


Figure 69. Expression of *PEG3* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

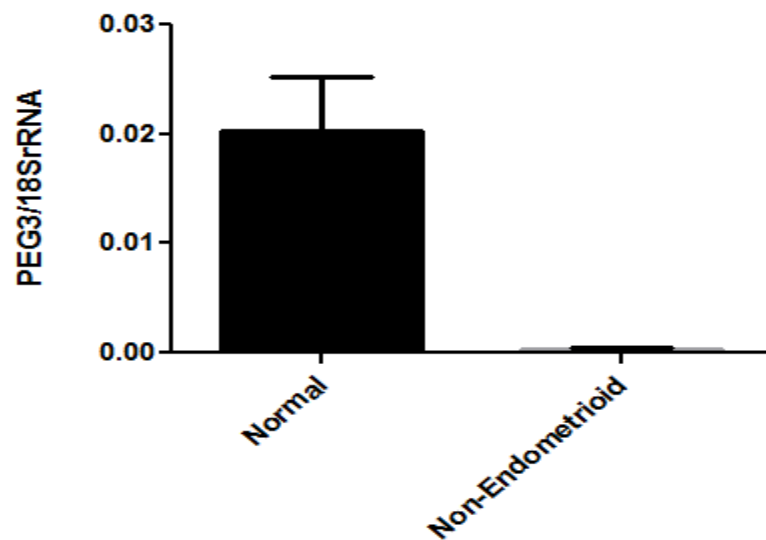


Figure 70. Expression of *PEG3* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

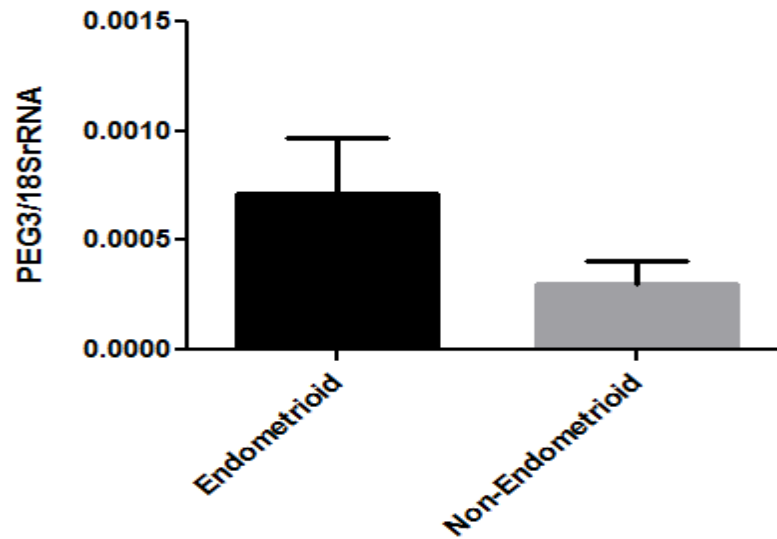


Figure 71. Expression of *PEG3* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Endometrioid and non-endometrioid tumors have comparable expression (p = 0.2108).

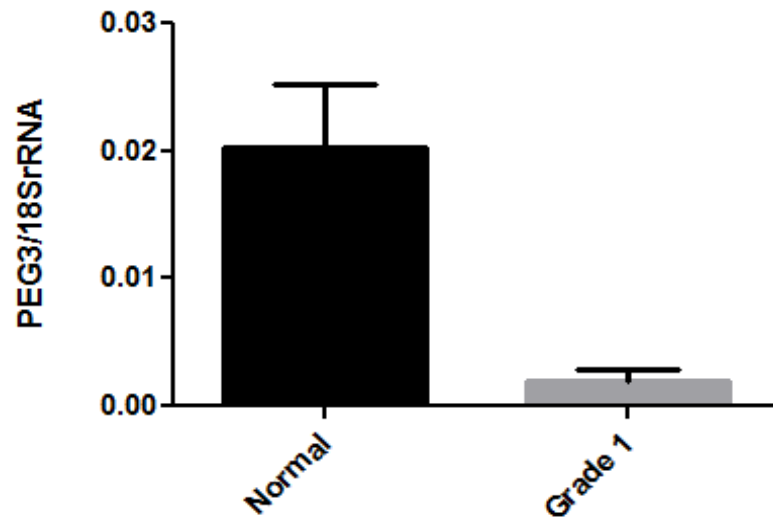


Figure 72. Expression of *PEG3* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Grade 1 tumors have significantly lower expression (p = 0.0030).

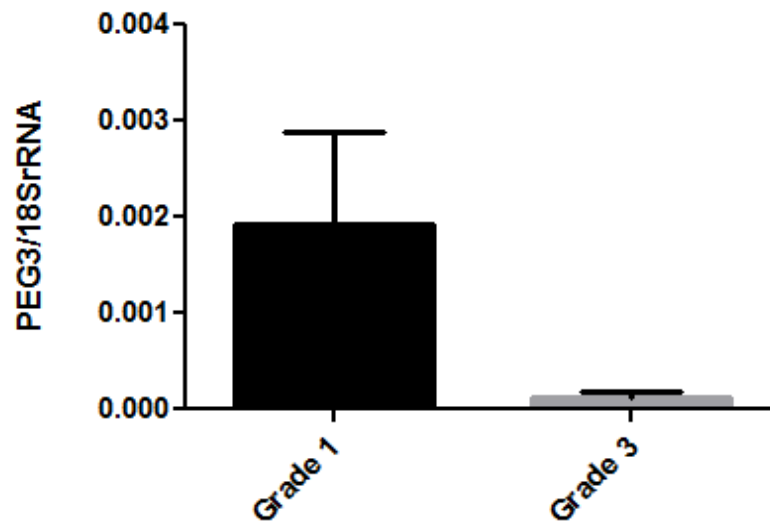


Figure 73. Expression of *PEG3* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0335).

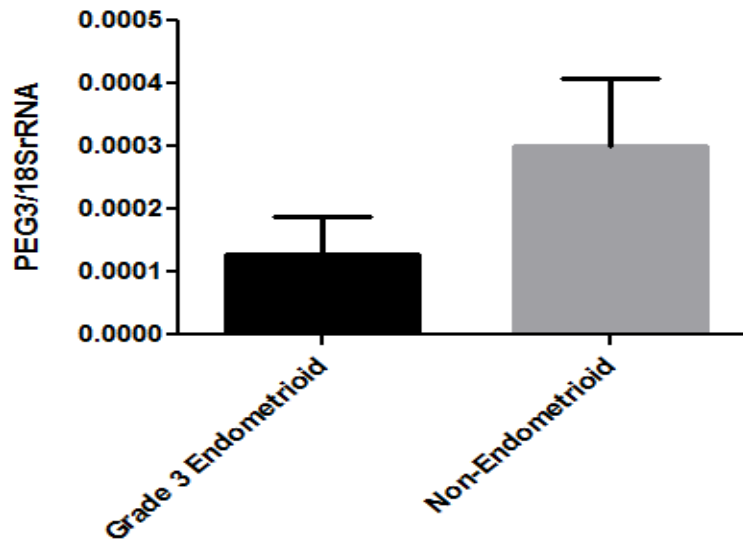


Figure 74. Expression of *PEG3* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significantly different (p = 0.2702).

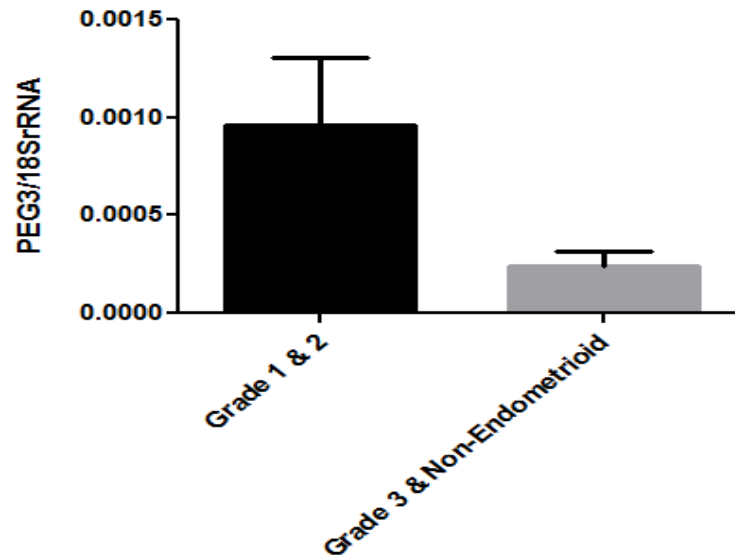


Figure 75. Expression of *PEG3* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0203).

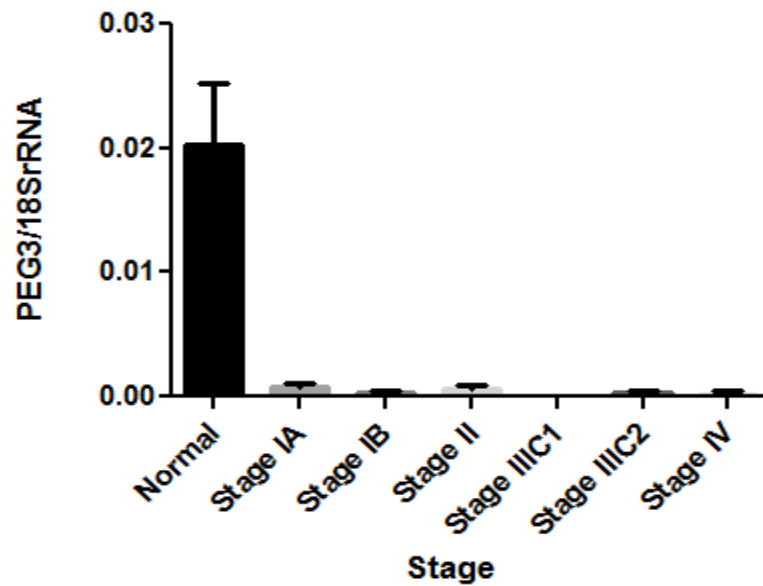


Figure 76. Expression of *PEG3* in normal endometrium and in endometrial carcinomas by FIGO stage.

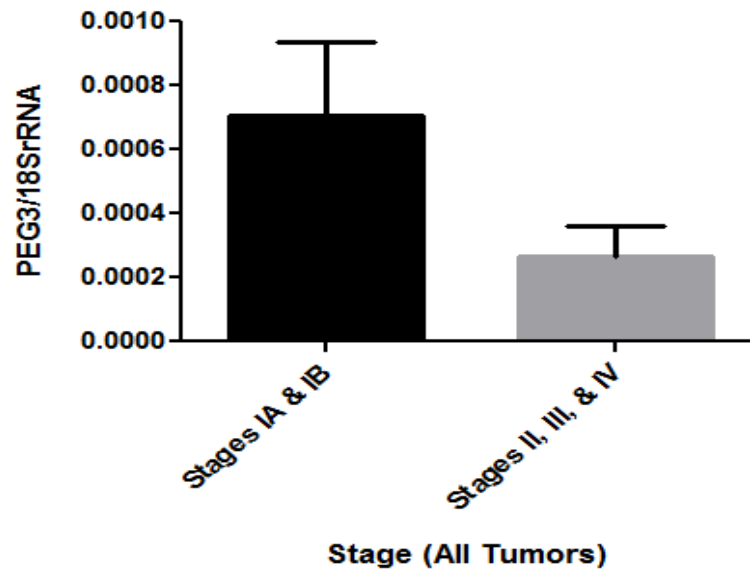


Figure 77. Expression of *PEG3* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.1930).

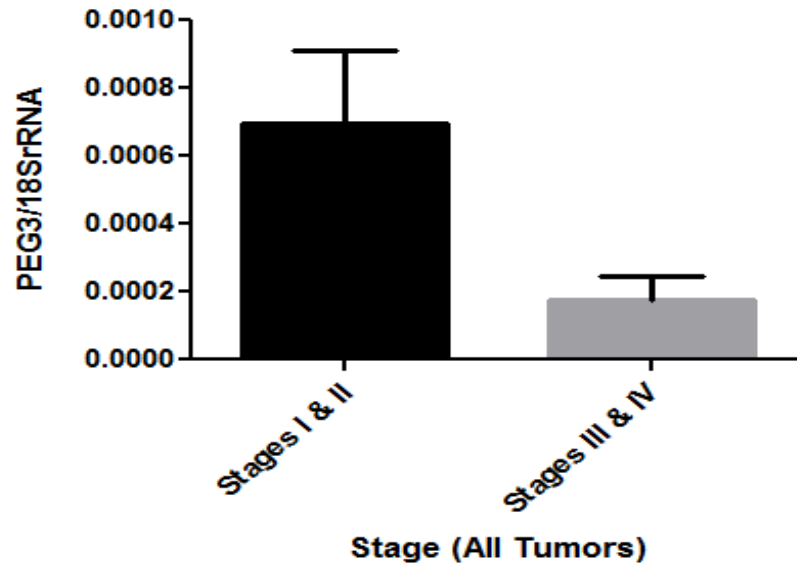


Figure 78. Expression of *PEG3* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.1533).

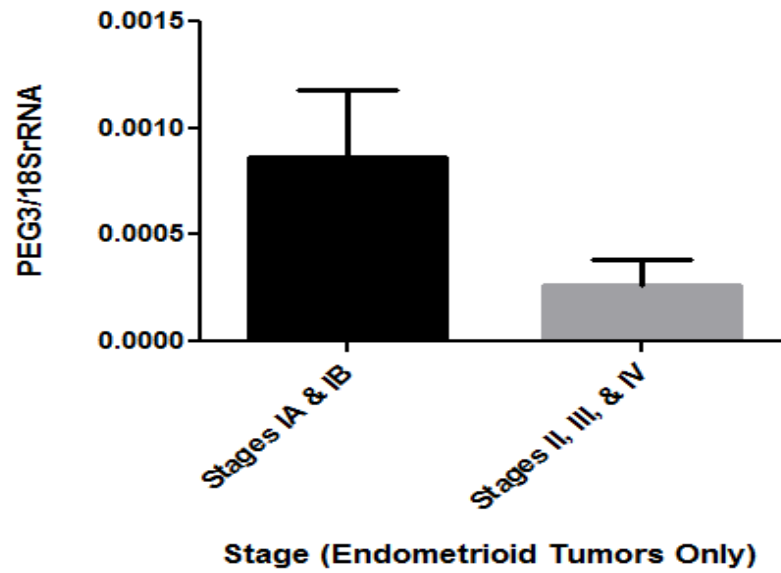


Figure 79. Expression of *PEG3* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.3020$).

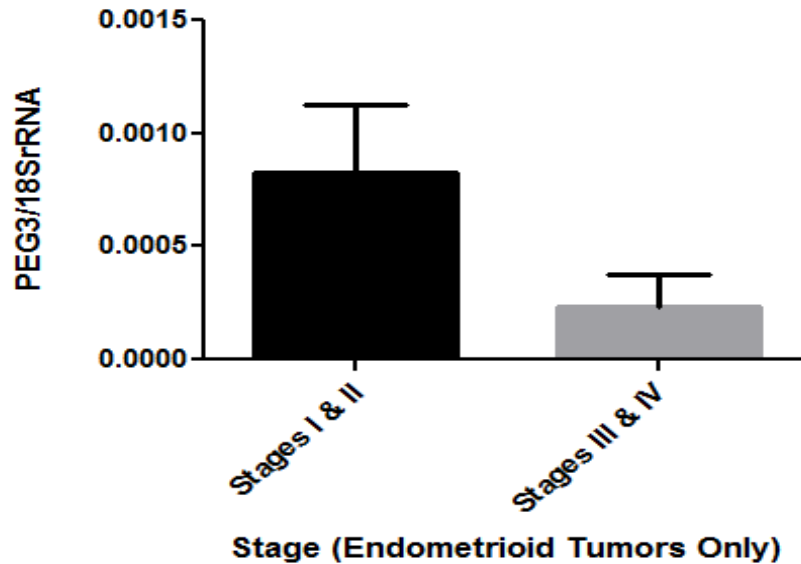


Figure 80. Expression of *PEG3* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.3486$).

PEG3 gene expression was significantly lower in endometrial carcinomas compared to normal endometrium. *PEG3* expression levels decreased in correlation with increasing FIGO grade ($p = <0.0001$). Our results are in accordance with literature, with others finding *PEG3* expression to be present in normal endometrial tissues, but no expression in endometrial cancer cell lines (19). This suggests *PEG3* acts as a tumor suppressor gene in endometrial cancers.

EFNA1

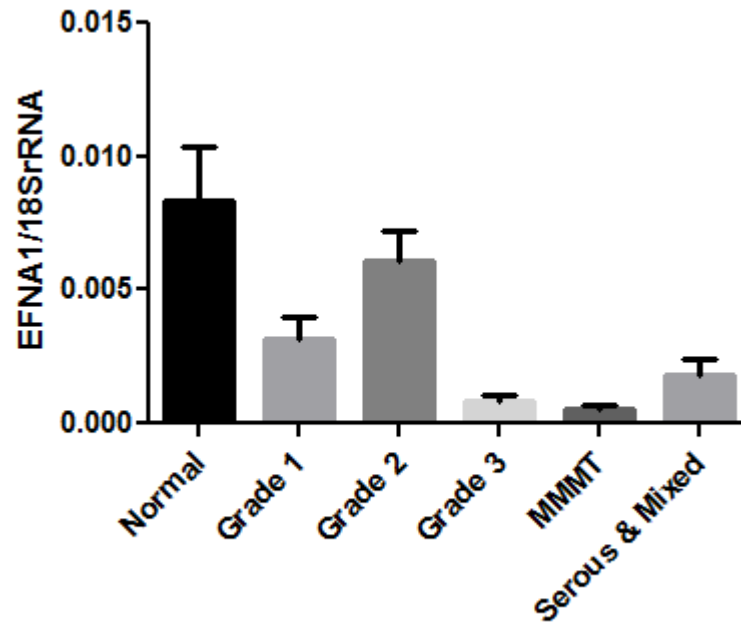


Figure 81. Expression of *EFNA1* in normal endometrium and in endometrial carcinomas. This graph compares *EFNA1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of *EFNA1* was decreased in carcinomas compared to normal, especially for the non-endometrioid carcinomas.

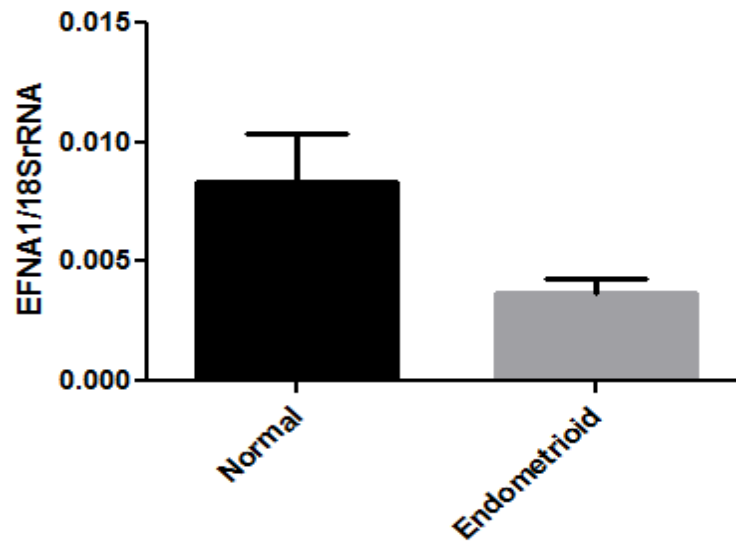


Figure 82. Expression of *EFNA1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is lower in the endometrioid tumors overall (p = 0.0072).

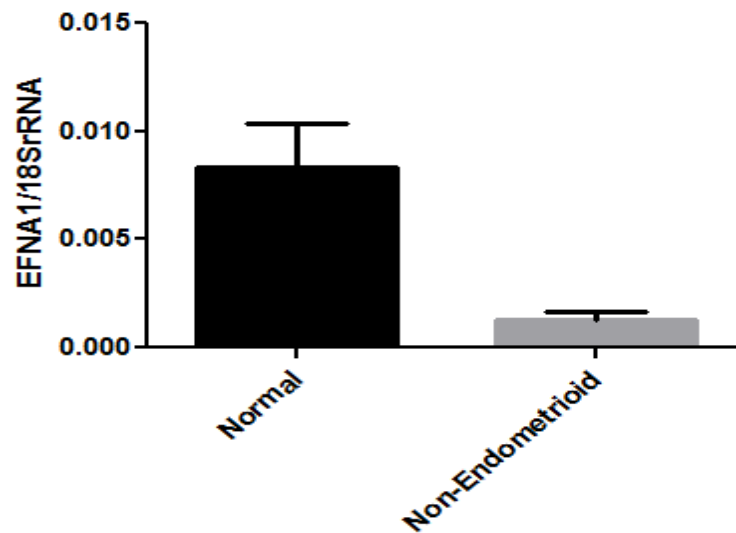


Figure 83. Expression of *EFNA1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

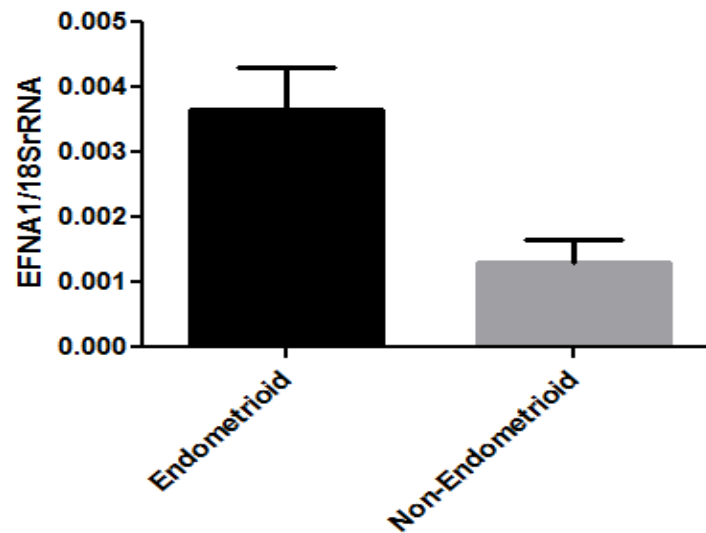


Figure 84. Expression of *EFNA1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0133).

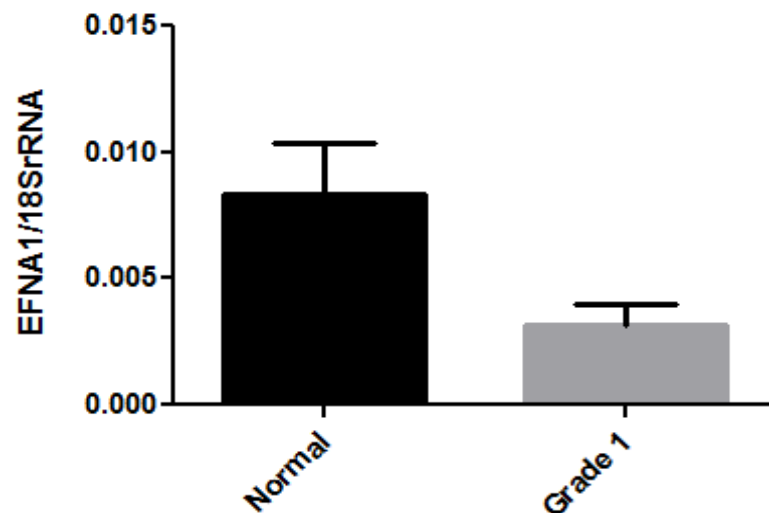


Figure 85. Expression of *EFNA1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Grade 1 tumors have significantly lower expression (p = 0.0315).

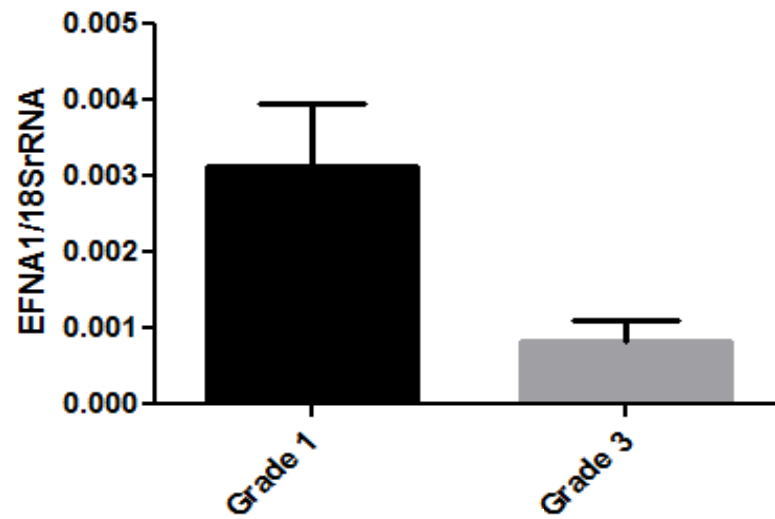


Figure 86. Expression of *EFNA1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0031).

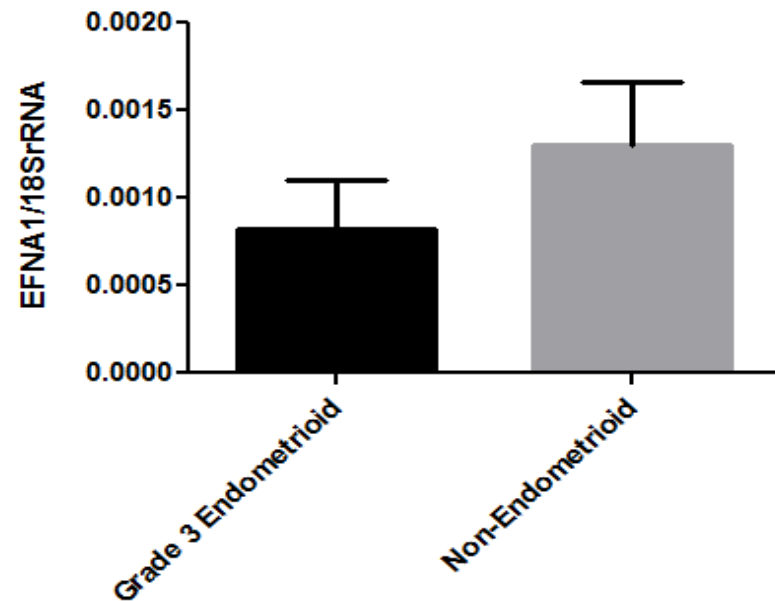


Figure 87. Expression of *EFNA1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is comparable (p = 0.3486).

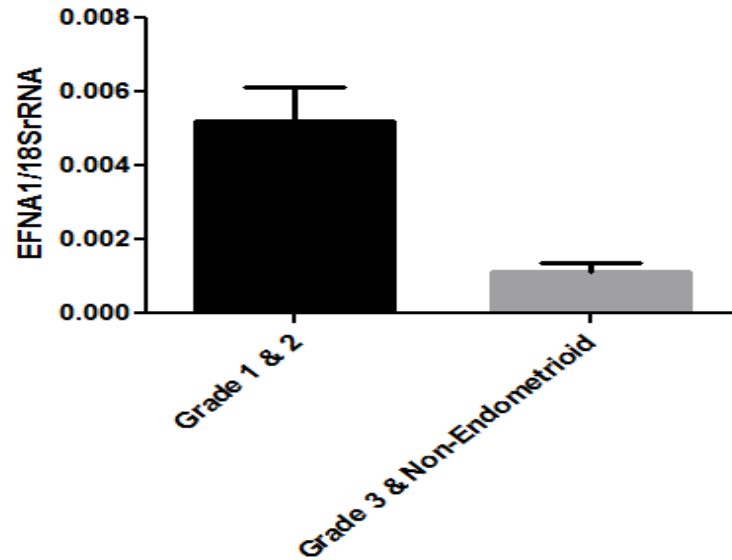


Figure 88. Expression of *EFNA1* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = <0.0001).

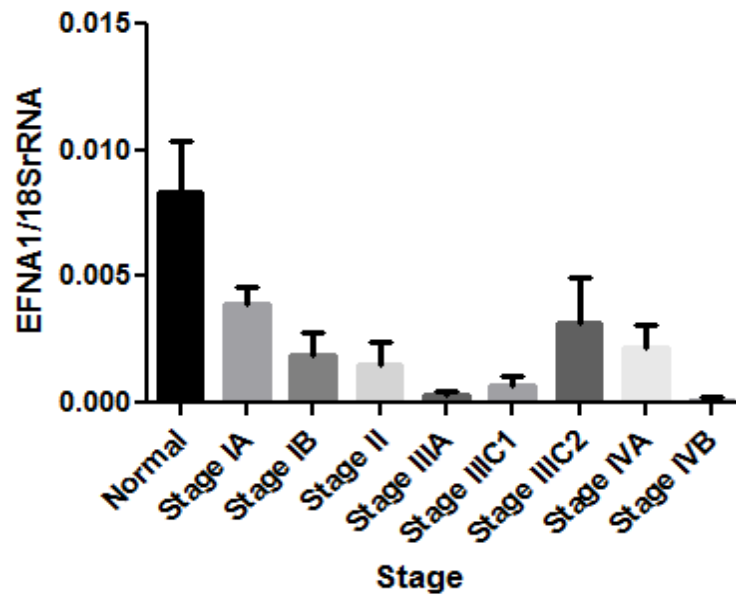


Figure 89. Expression of *EFNA1* in normal endometrium and in endometrial carcinomas by FIGO stage.

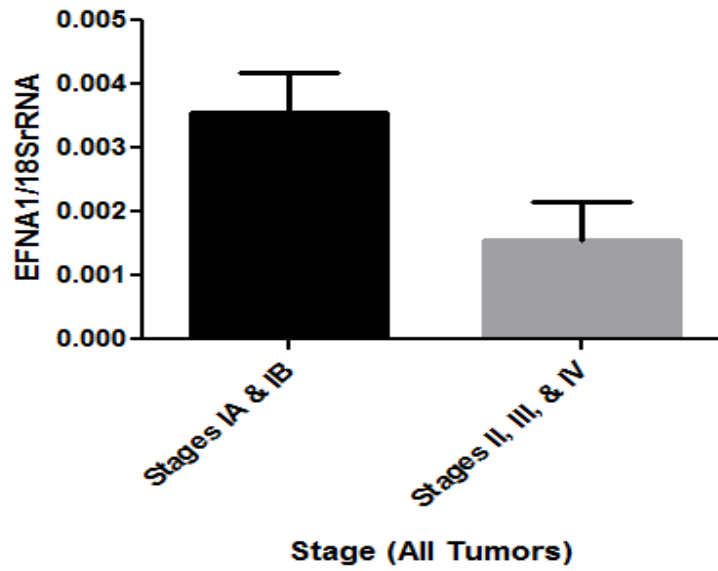


Figure 90. Expression of *EFNA1* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression (p = 0.0345).

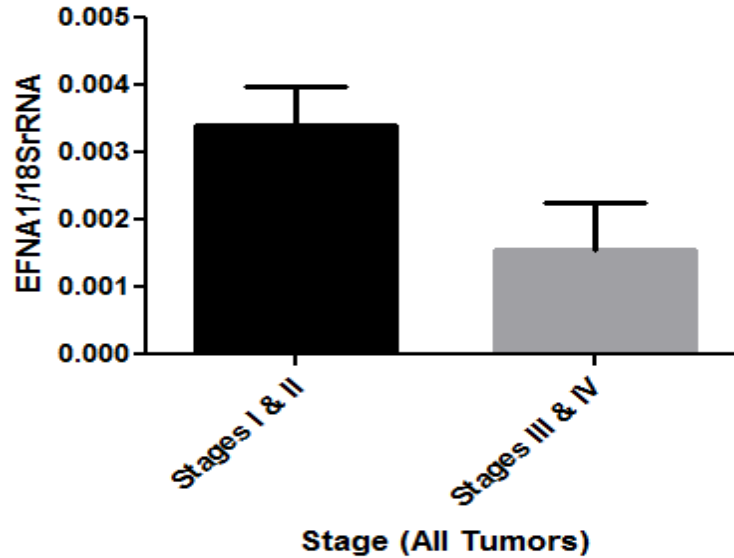


Figure 91. Expression of *EFNA1* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.0638).

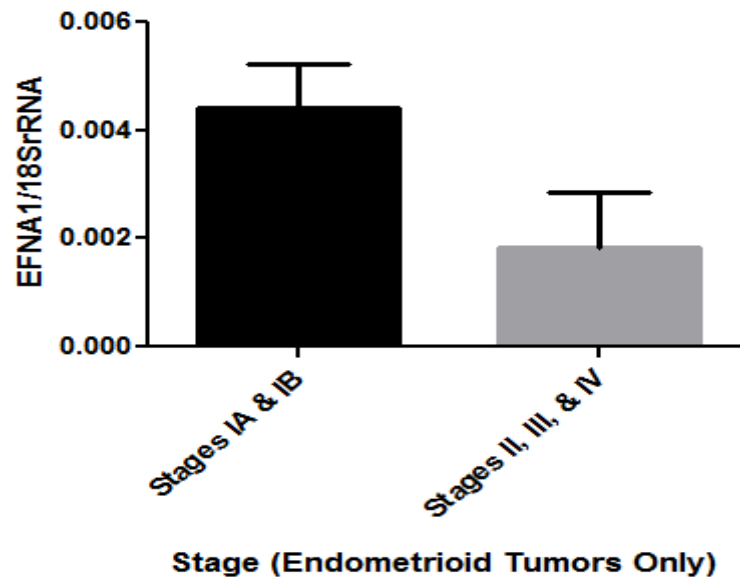


Figure 92. Expression of *EFNA1* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.0727$).

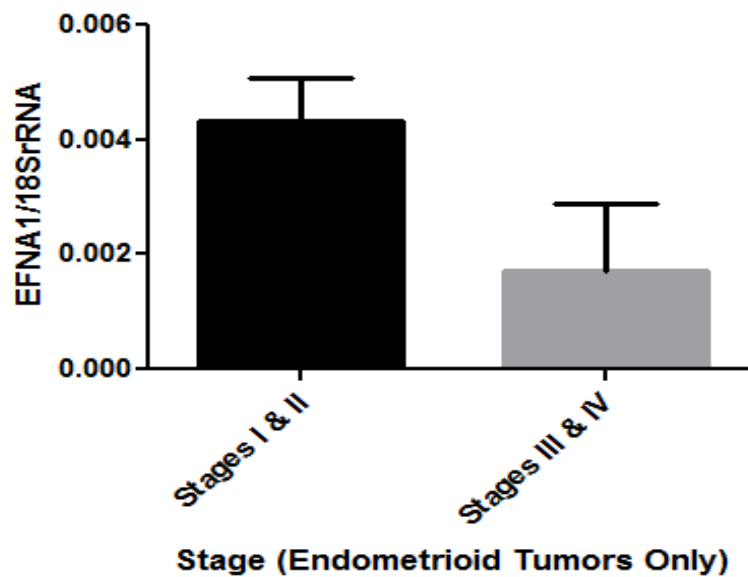


Figure 93. Expression of *EFNA1* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.0842$).

Our results demonstrated *EFNA1* expression to be significantly down-regulated in endometrioid and non-endometrioid carcinomas compared to normal endometrium. *EFNA1* expression has not been studied in endometrial cancers but it has been shown through qRT-PCR and immunohistochemistry to have an increase in expression in ovarian cancers, vulvar carcinomas, and melanomas (21). We found the opposite, suggesting *EFNA1* may have a tumor suppressive role in endometrial cancers.

EDNRA

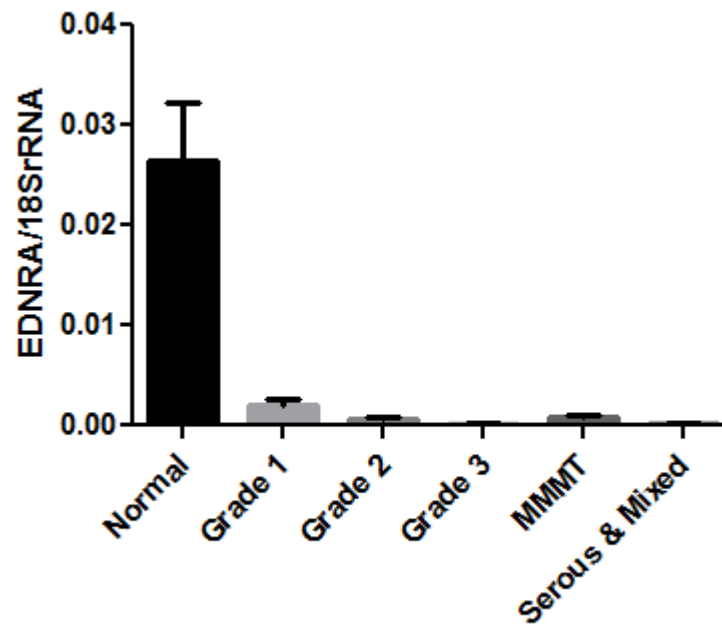


Figure 94. Expression of *EDNRA* in normal endometrium and in endometrial carcinomas. This graph compares *EDNRA* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression was significantly lower decreased in endometrial carcinomas compared to normal.

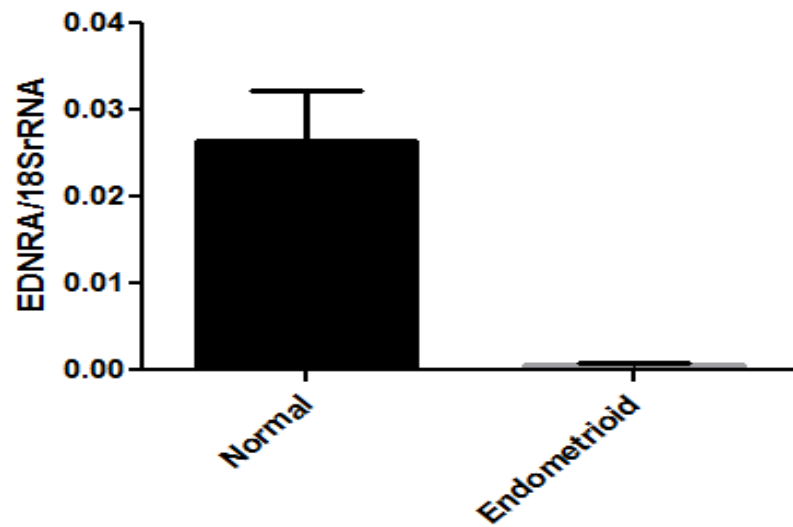


Figure 95. Expression of *EDNRA* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

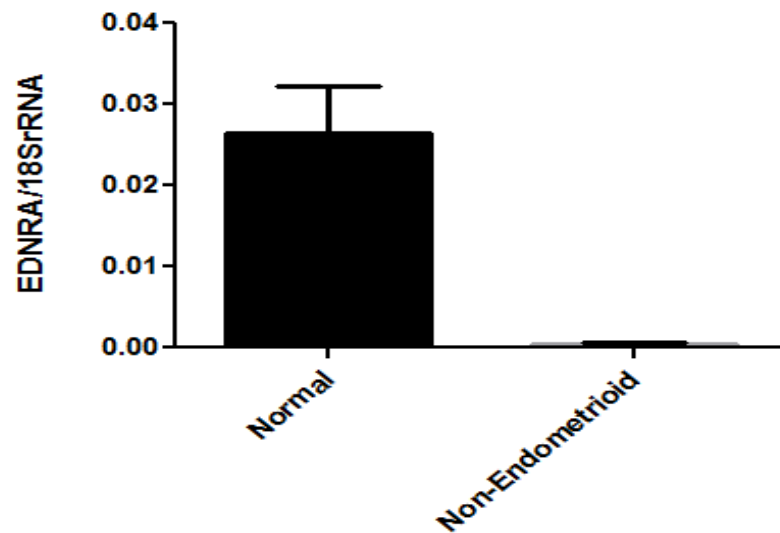


Figure 96. Expression of *EDNRA* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

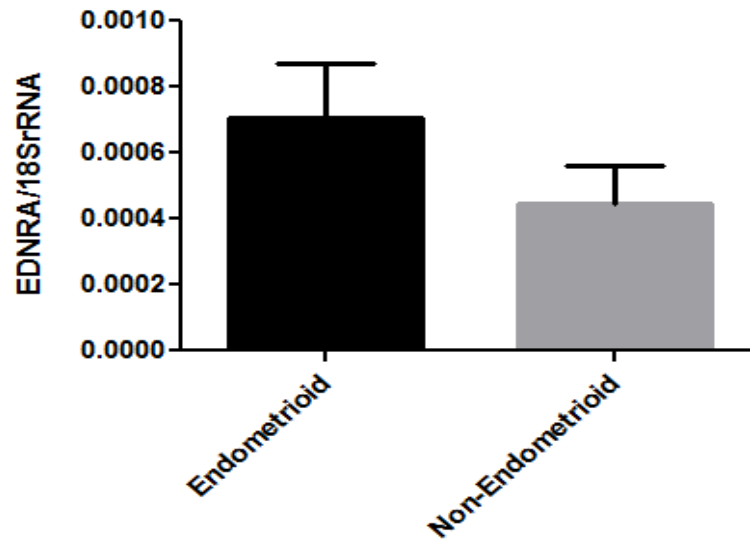


Figure 97. Expression of *EDNRA* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Endometrioid and non-endometrioid tumors have comparable expression (p = 0.2655).

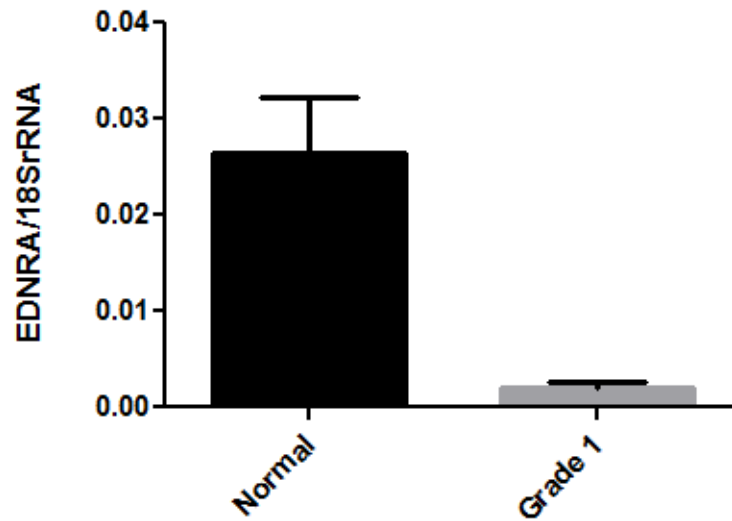


Figure 98. Expression of *EDNRA* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Grade 1 tumors have significantly lower expression (p = 0.0020).

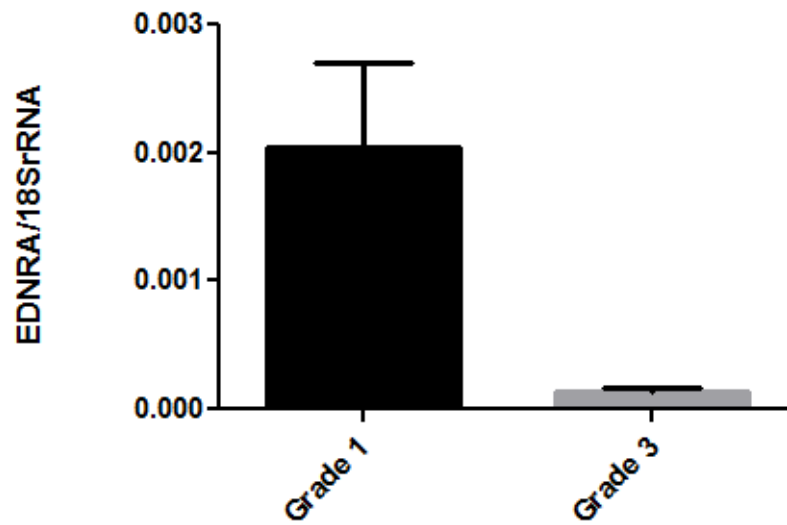


Figure 99. Expression of *EDNRA* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0004).

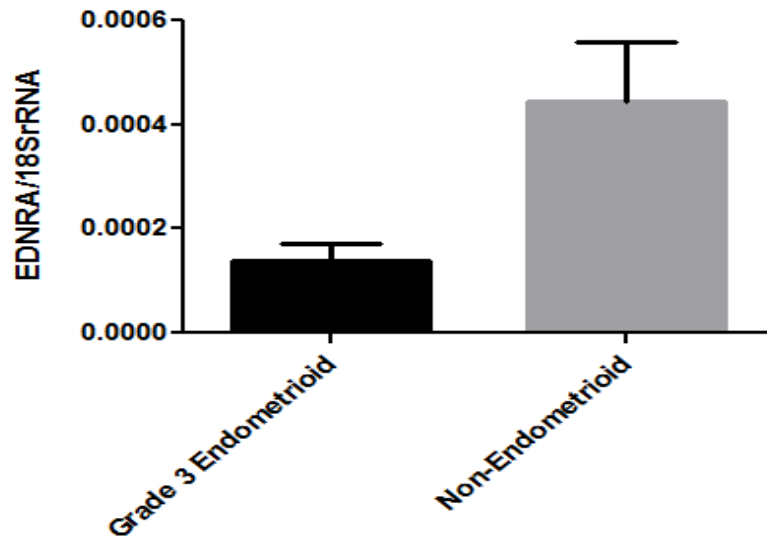


Figure 100. Expression of *EDNRA* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression is slightly higher in non-endometrioid tumors (p = 0.0462).

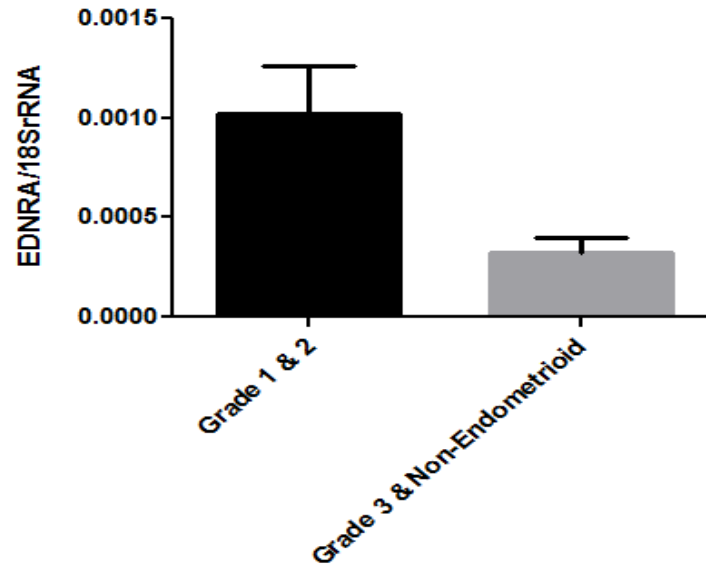


Figure 101. Expression of *EDNRA* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.002).

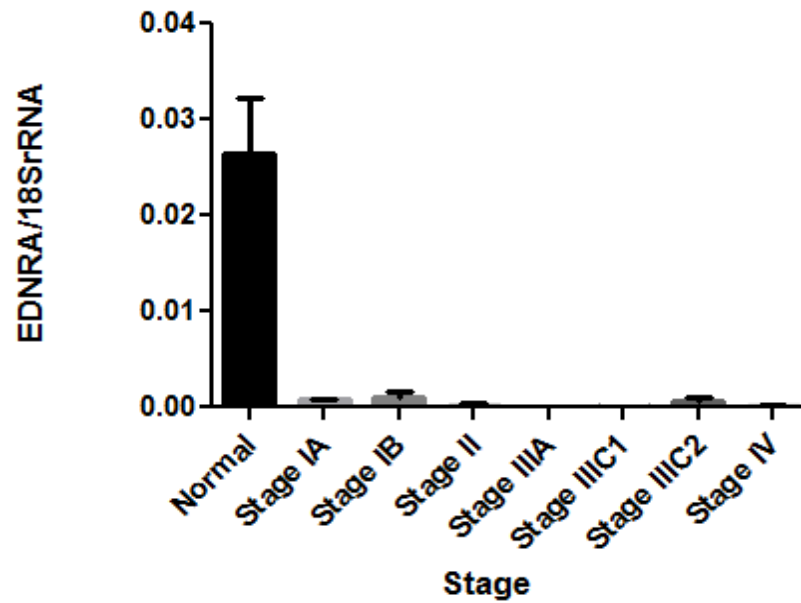


Figure 102. Expression of *EDNRA* in normal endometrium and in endometrial carcinomas by FIGO stage.

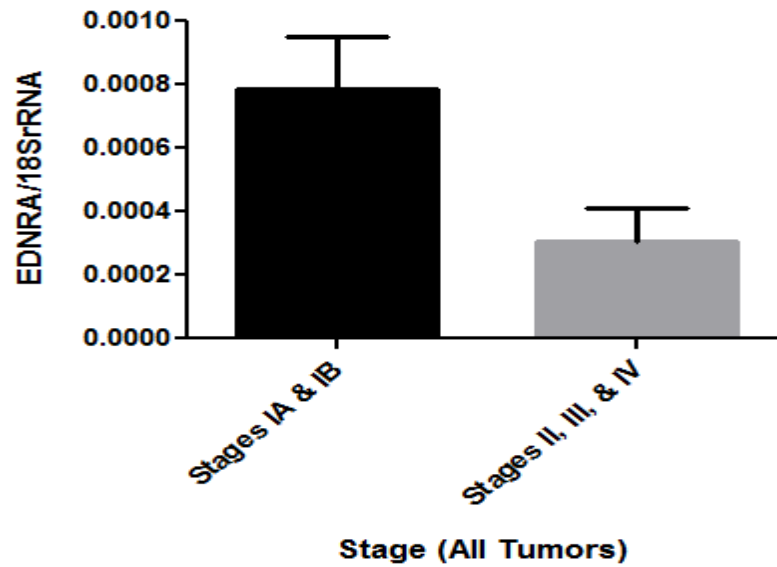


Figure 103. Expression of *EDNRA* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression ($p = 0.0392$).

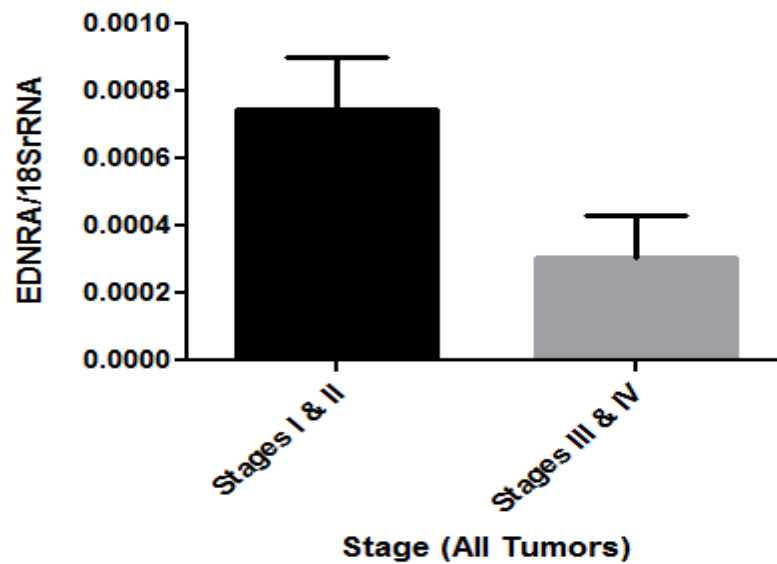


Figure 104. Expression of *EDNRA* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant ($p = 0.0700$).

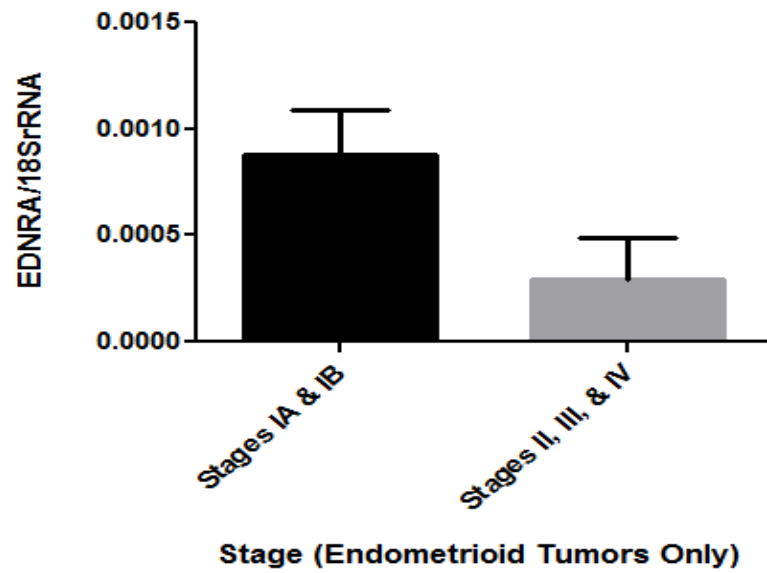


Figure 105. Expression of *EDNRA* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.1103$).

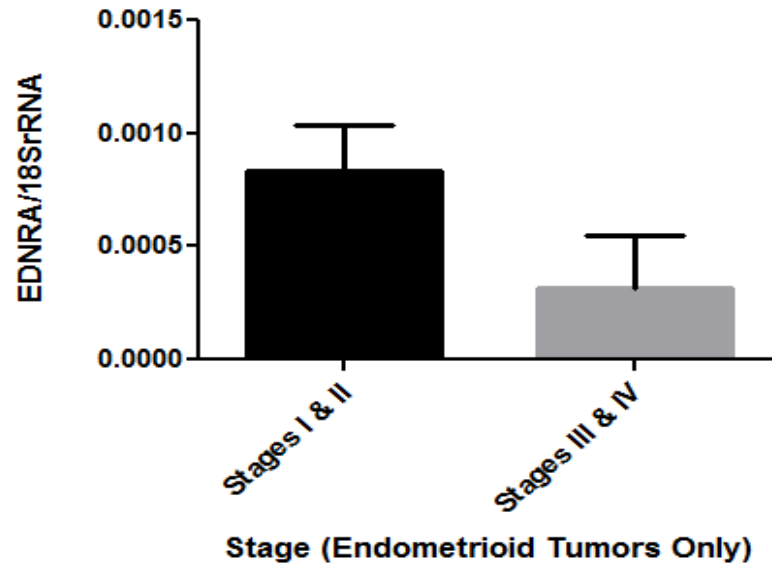


Figure 106. Expression of *EDNRA* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.1814$).

There was a strong down-regulation of *EDNRA* expression in both endometrioid and non-endometrioid carcinomas compared to normal endometrial tissues. This gene has not been previously studied in endometrial cancers, but it has been found to have higher expression levels through immunohistochemistry in grades 3 and 4 ovarian serous and endometrioid carcinomas compared to low grade ovarian carcinomas (23), with 52% of high grade ovarian cancers showing positive staining for *EDNRA*.

HEYL

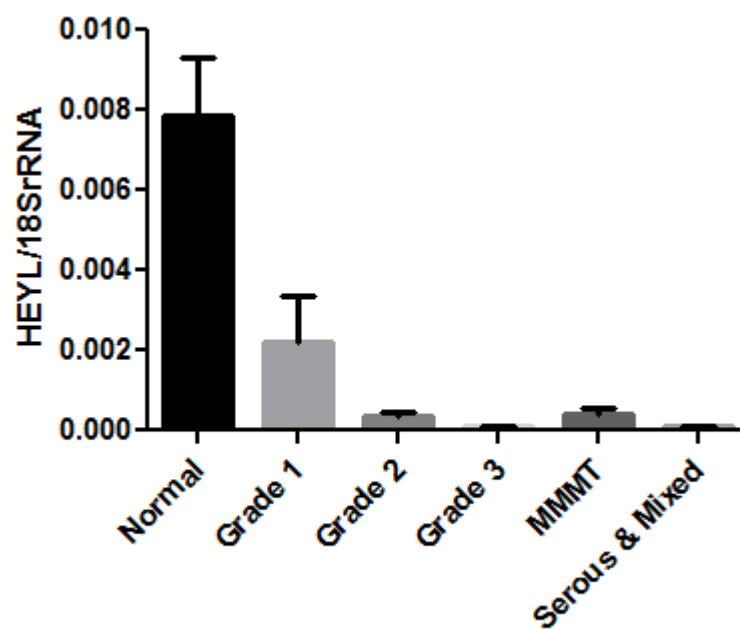


Figure 107. Expression of *HEYL* in normal endometrium and in endometrial carcinomas. This graph compares *HEYL* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression was significantly decreased in endometrial carcinomas compared to normal.

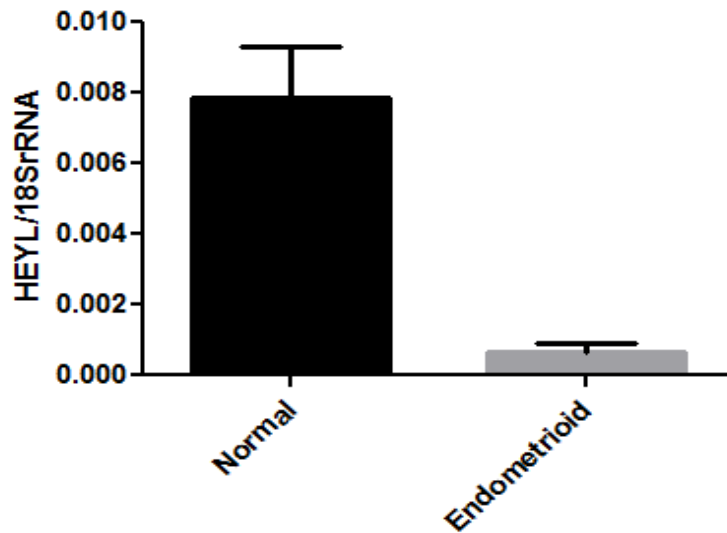


Figure 108. Expression of *HEYL* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall ($p = <0.0001$).

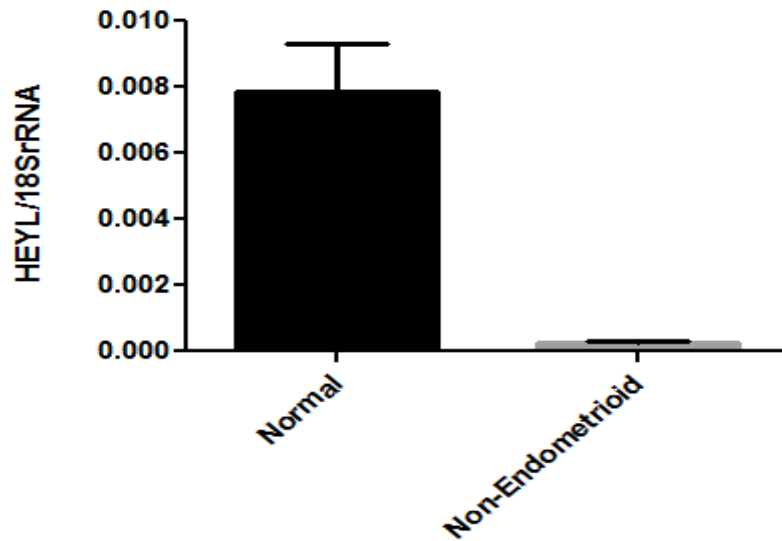


Figure 109. Expression of *HEYL* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression ($p = <0.0001$).

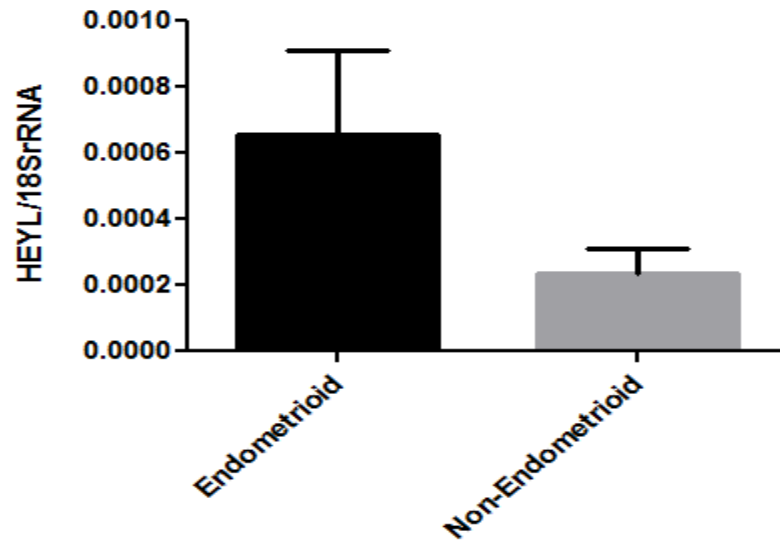


Figure 110. Expression of *HEYL* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Endometrioid and non-endometrioid tumors have comparable expression (p = 0.1940).

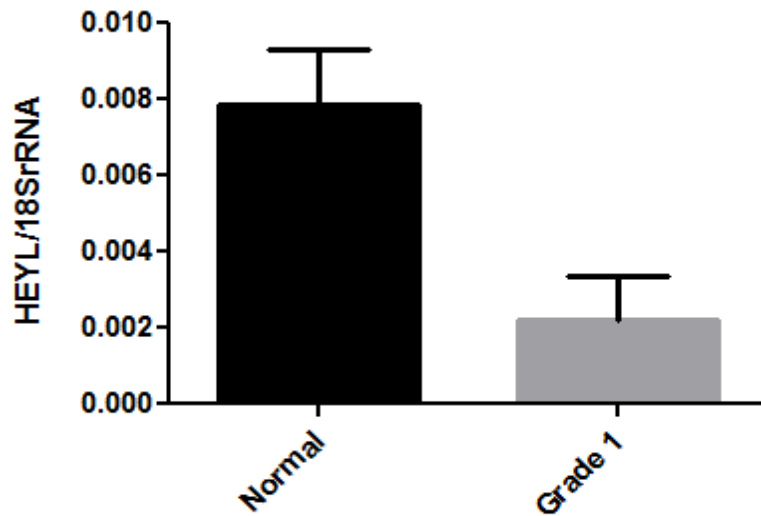


Figure 111. Expression of *HEYL* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Grade 1 tumors have significantly lower expression (p = 0.0122).

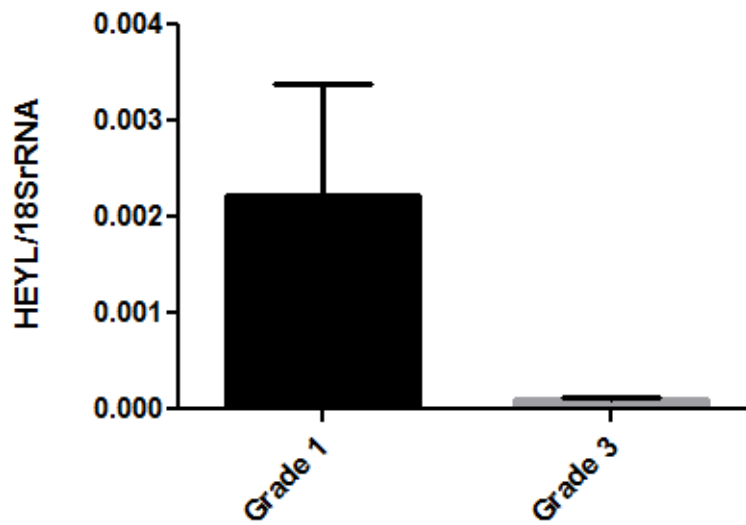


Figure 112. Expression of *HEYL* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression than grade 1 tumors (p = 0.0397).

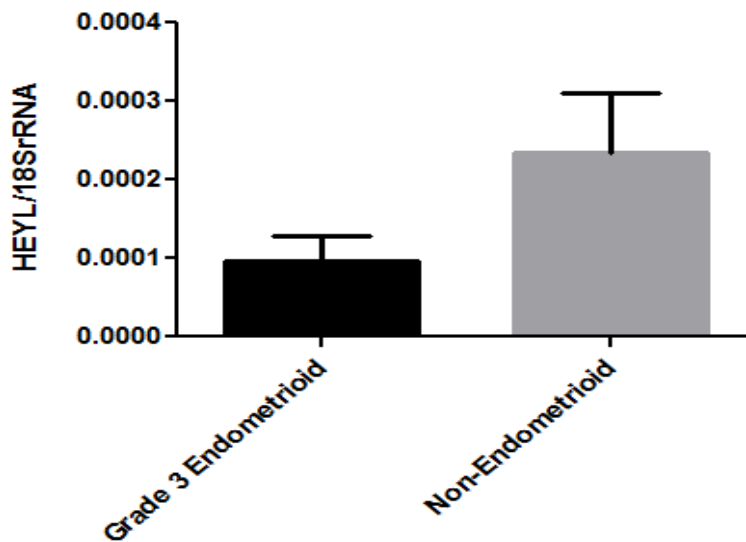


Figure 113. Expression of *HEYL* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significant (p = 0.2551).

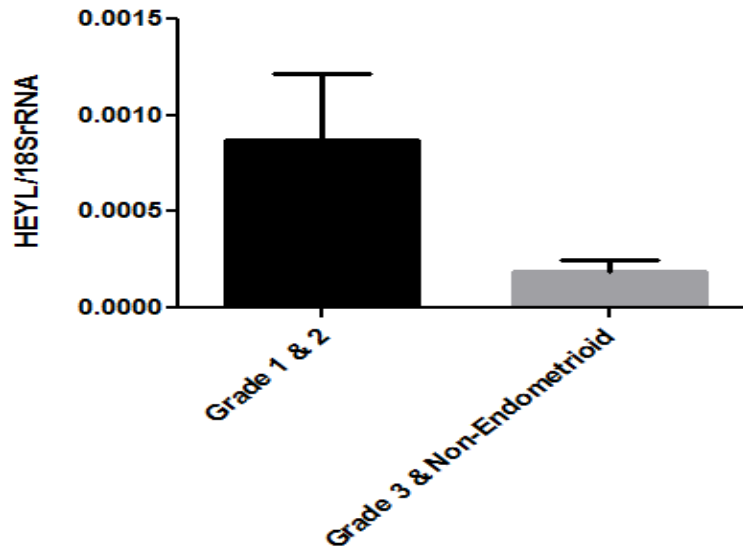


Figure 114. Expression of *HEYL* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0311).

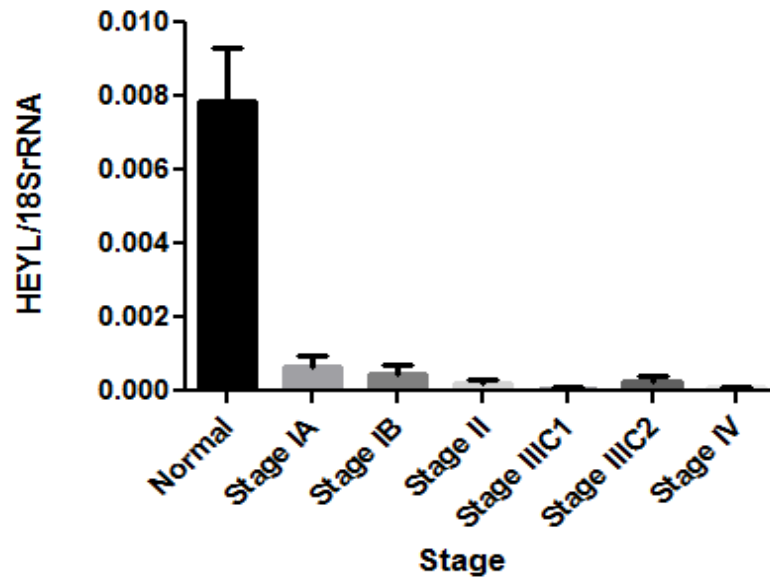


Figure 115. Expression of *HEYL* in normal endometrium and in endometrial carcinomas by FIGO stage.

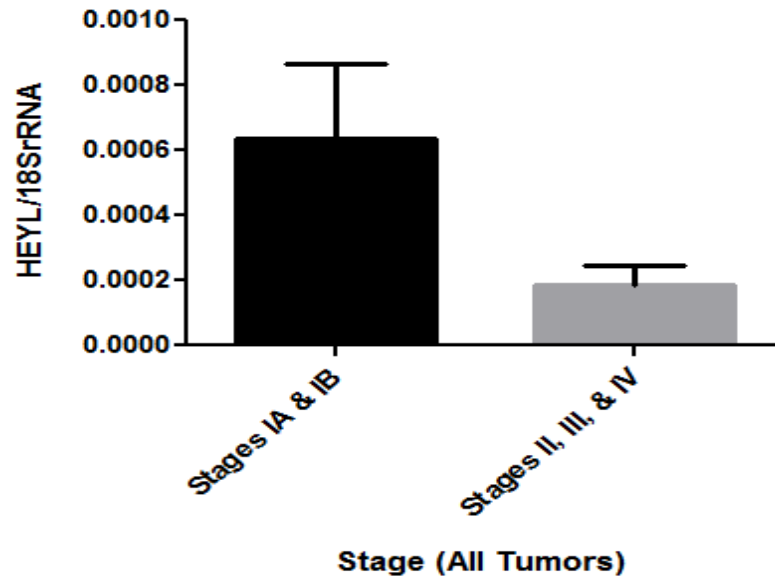


Figure 116. Expression of *HEYL* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have slightly lower expression, but this difference is not significant (p = 0.1831).

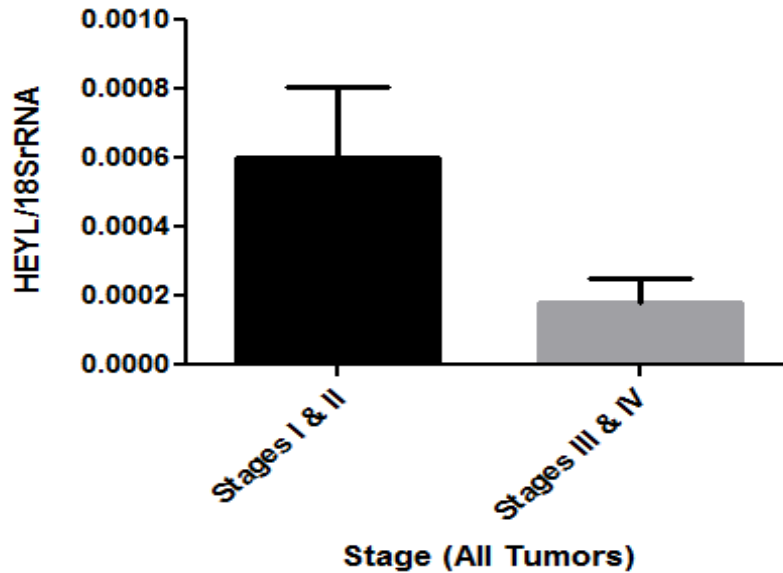


Figure 117. Expression of *HEYL* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.2535).

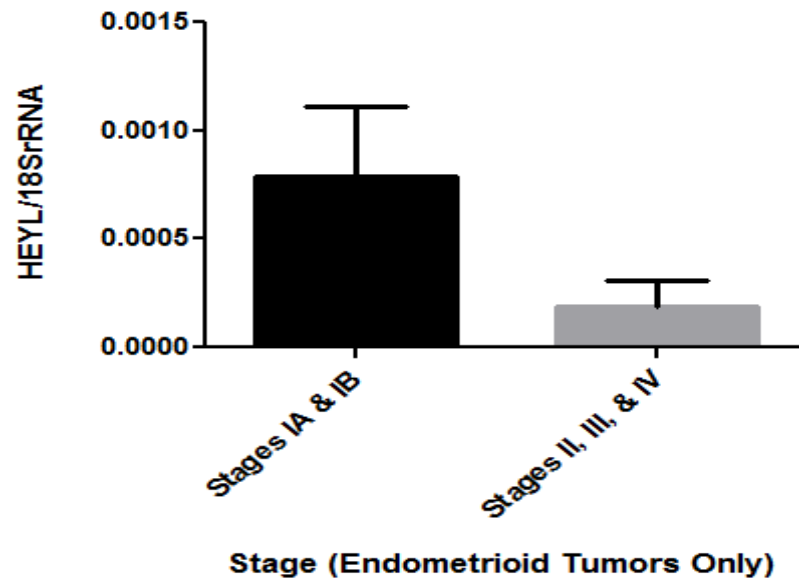


Figure 118. Expression of *HEYL* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.3269$).

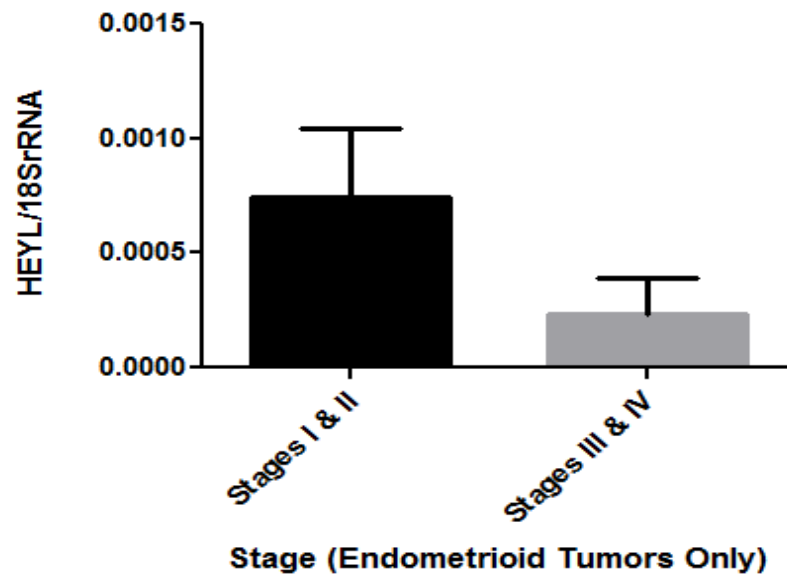


Figure 119. Expression of *HEYL* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.4558$).

Our results showed *HEYL* expression to be significantly lower in grade 1 endometrioid endometrial carcinomas compared to normal endometrium ($p = 0.0122$). Expression levels decrease in accordance with increasing grade ($p = <0.0001$). The opposite has been observed in other cancers. For examples, *HEYL* expression has been found to be overexpressed in the more clinically invasive breast cancers. Parker et al. (25) used a *HEYL* mixture probe to test for *HEYL* expression in normal breast tissues and in invasive ductal carcinomas. They did not find any staining in the normal tissues, but there was a strong labeling of the *HEYL* probes in invasive ductal carcinomas.

HES1

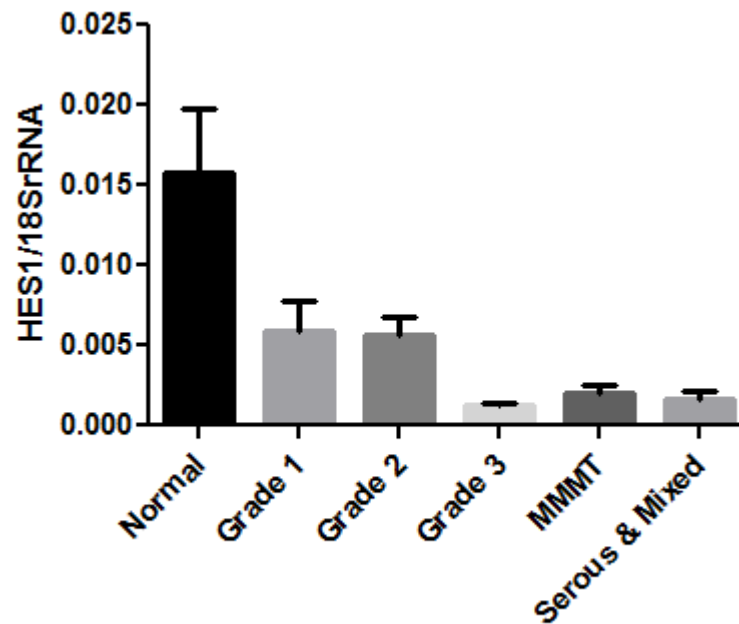


Figure 120. Expression of *HES1* in normal endometrium and in endometrial carcinomas. This graph compares *HES1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression is significantly decreased in endometrial carcinomas compared to normal.

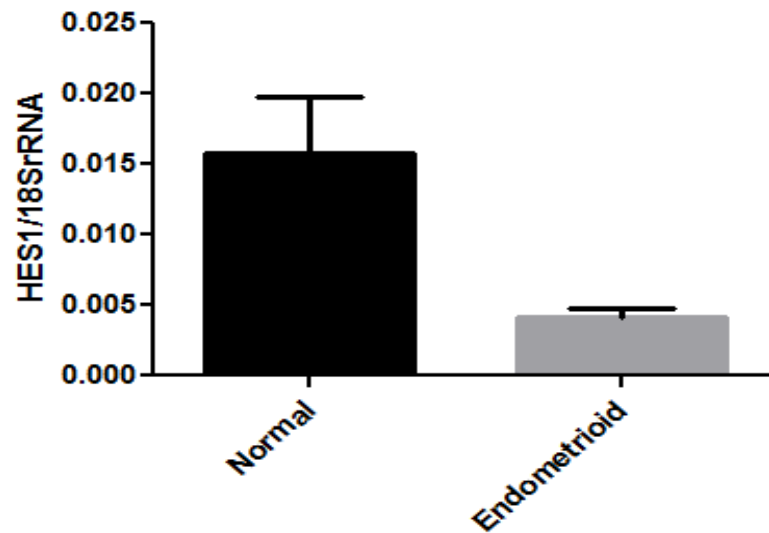


Figure 121. Expression of *HES1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

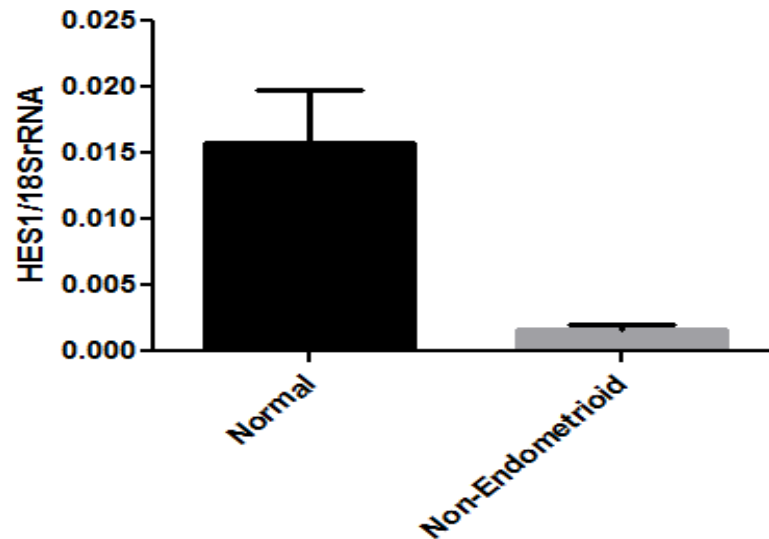


Figure 122. Expression of *HES1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

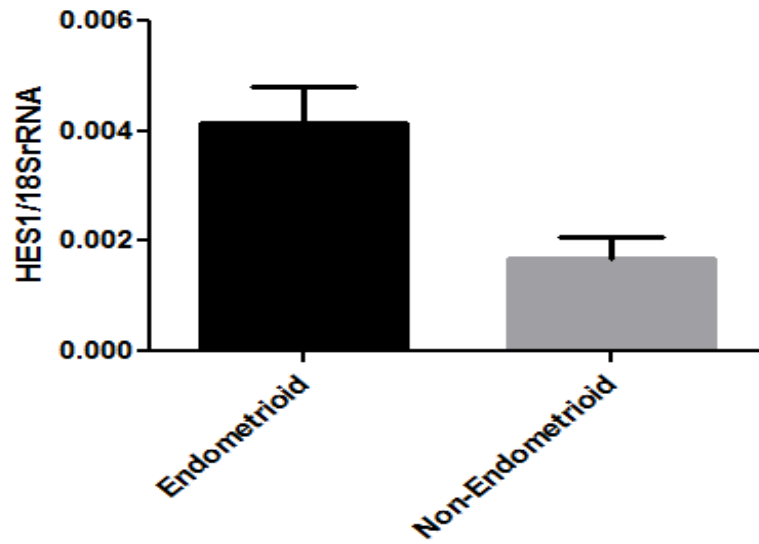


Figure 123. Expression of *HES1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0247).

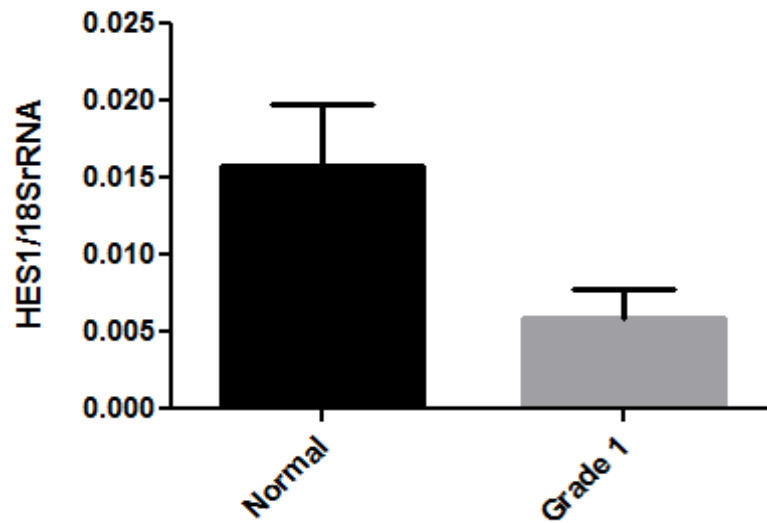


Figure 124. Expression of *HES1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Expression is significantly lower in grade 1 tumors (p = 0.0435).

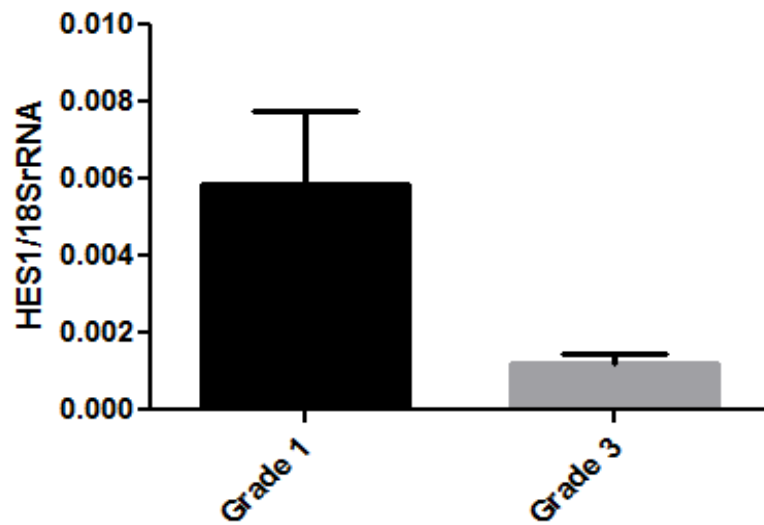


Figure 125. Expression of *HES1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression (p = 0.0030).

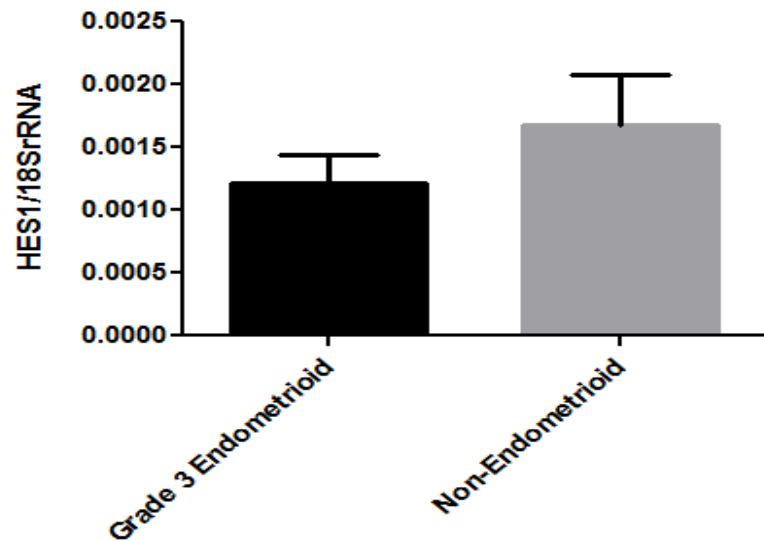


Figure 126. Expression of *HES1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significant (p = 0.3364).

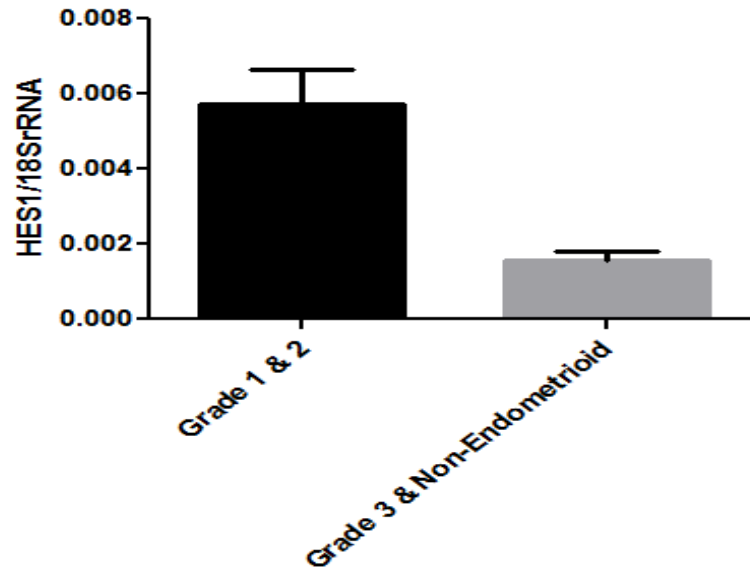


Figure 127. Expression of *HES1* in grades 1 and 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = <0.0001).

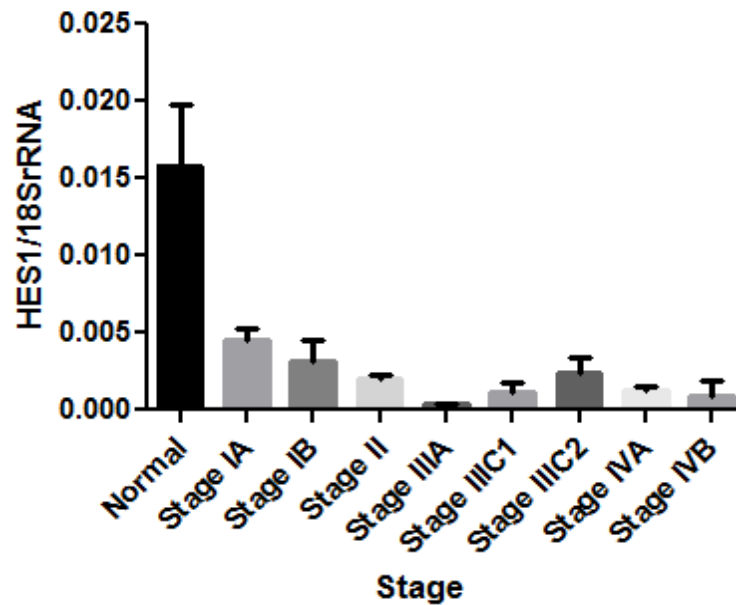


Figure 128. Expression of *HES1* in normal endometrium and in endometrial carcinomas by FIGO stage.

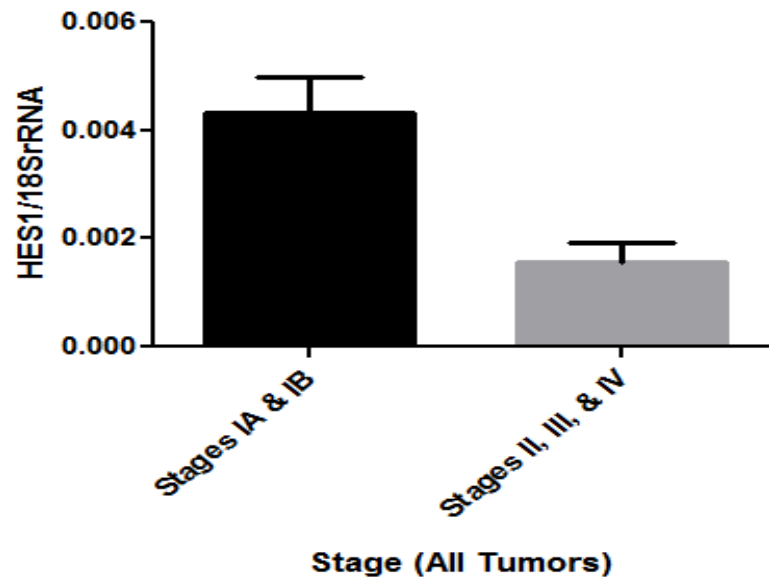


Figure 129. Expression of *HES1* in stages IA and IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression (p = 0.0039).

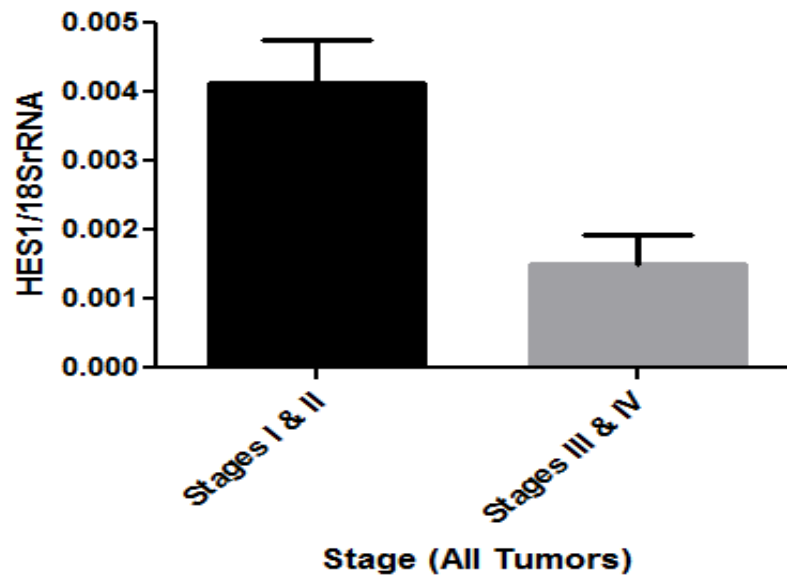


Figure 130. Expression of *HES1* in stage I and II endometrial carcinomas and in stages III and IV endometrial carcinomas. Higher stages have significantly lower expression (p = 0.0081).

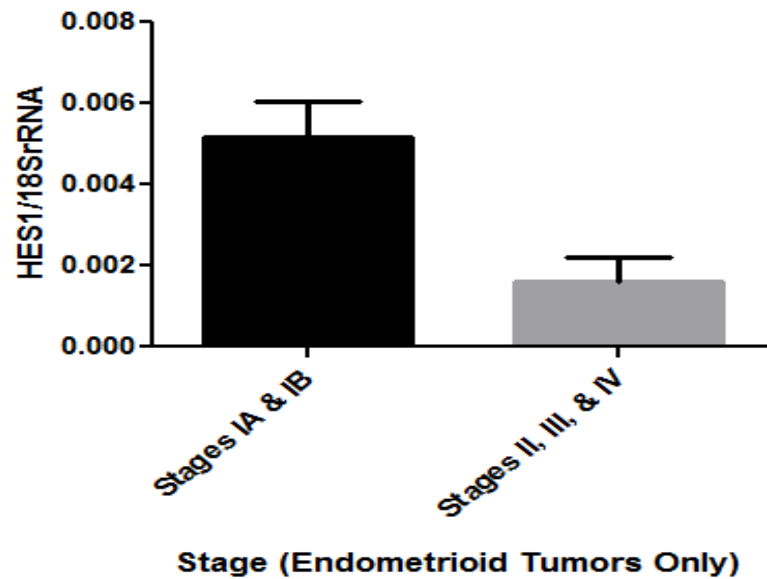


Figure 131. Expression of *HES1* in stages IA and IB endometrioid endometrial carcinomas compared to higher stages combined. Higher stages have significantly lower expression ($p = 0.0130$).

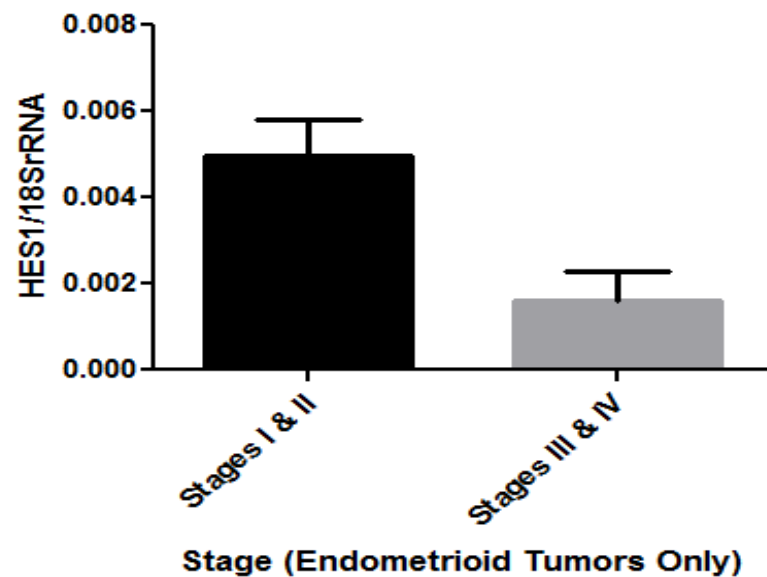


Figure 132. Expression of *HES1* in stages I and II endometrioid endometrial carcinomas compared to higher stages. Higher stages have significantly lower expression ($p = 0.0260$).

Similar to *HEYL*, *HES1* expression was significantly higher in normal endometrial tissues compared to grade 1 endometrioid carcinomas. In ovarian cancers, *HES1* is overexpressed. Hopfer et al. (27) studied *HES1* expression levels in ovarian epithelial adenocarcinomas, ovarian adenomas, and epithelial ovarian cancers of low malignant potential, as well as human ovarian adenocarcinoma cell lines. Through qRT-PCR and Western blot analysis, *HES1* expression was found to be higher in the malignant tumors compared to the benign tumors. Expression was intermediate in 11 out of 19 invasive adenocarcinomas and strong in 7 out of 19 invasive adenocarcinomas. (27)

Table 6 provides a summary of which genes in this panel may play a role in important clinical/pathological distinctions for endometrial carcinoma. Note that many of the genes might be potentially useful in distinguishing normal endometrium from grade 1 endometrioid carcinomas. This may be helpful in clinical cancer prevention trials. Additionally, expression of many of the genes is significantly lower in grade 3 endometrioid carcinomas compared to grade 1 endometrioid carcinomas. Grade 3 endometrioid tumors are more often clinically aggressive. Interestingly, only one gene in this panel (*HES1*) has potential utility in predicting stage. This suggests that strategies to identify endometrial cancer biomarkers that rely on identifying normal vs. cancer differences may not be appropriate for finding endometrial cancer biomarkers predictive of stage.

Table 6. Summary of Gene Validation Results¹.

	Normal vs. Grade 1 Endometrioid	Endometrioid vs. Non- endometrioid	Grade 1 Endometrioid vs. Grade 3 Endometrioid	Stages I and II vs. Stages III and IV (Endometrioid tumors)
<i>P-cadherin</i>		X		
<i>OLFM1</i>	X		X	
<i>VAV3</i>		X		
<i>SPRY1</i>	X		X	
<i>PIK3R1</i>		X	X	
<i>PEG3</i>	X		X	
<i>EFNA1</i>	X	X	X	
<i>EDNRA</i>	X		X	
<i>HEYL</i>	X		X	
<i>HES1</i>	X	X	X	X

¹The X indicates scenarios in which a specific gene may be useful in discriminating one group from another.

Chapter 4

Notch Pathway Introduction

From Chapter 3, two of the genes analyzed, *HEYL* and *HES1*, are known downstream target genes of the Notch signaling pathway. Both of these genes were significantly down-regulated in endometrial carcinomas (endometrioid and non-endometrioid histotypes) compared to normal endometrium. Therefore, we were interested in probing the Notch pathway in more detail.

The Notch signaling pathway significantly contributes to the pathogenesis of various cancers. Depending on the cell type, it can have a tumor suppressive role, as well as an oncogenic/growth promoting role (29, 30, 31). The Notch pathway is comprised of many components, including ligands, receptors, transcription factors, and target genes (29, 30, 31). Figure 133 is a schematic of this pathway, which is described in detail below.

There are five Notch ligands, Delta-like ligand (Dll) 1, 3, 4, and Jagged (JAG) 1 and 2. They are single transmembrane proteins containing an extracellular Delta-Serrate-Lag-2 (DSL) domain that mediates receptor binding, and multiple EGF-like repeats. Jagged ligands have an extra cysteine-rich domain which Delta like ligands do not have. There are four Notch receptors, Notch 1-4. These receptors are single protein precursors that are cleaved during their transport to the cell surface by a furin-like protease. The receptors are heterodimers which contain an extracellular domain and an intracellular domain. (29, 30)

All four Notch receptors use the same general signaling pathway, which is activated by binding of one of the Notch ligands to the extracellular domain of a Notch receptor. The ligand-receptor complex then undergoes several proteolytic cleavages. The first cleavage is

facilitated by the ADAM/TACE family of proteases and produces NEXT (notch extracellular truncation). NEXT is then cleaved by the γ -secretase complex, which consists of two proteins, presenilin and nicastrin (29). This cleavage is a critical step as it releases the intracellular domain of Notch, NICD. NICD subsequently translocates into the nucleus and binds to CSL, a constitutive transcriptional repressor. Upon Notch binding, CSL becomes a transcriptional activator and along with other co-factors, such as mastermind-like (MAML) proteins, induces transcription of downstream Notch target genes. There are several downstream targets of the Notch pathway, including the Hairy/Enhancer of Split related genes, Hes and Hey. (29, 30)

Hes and Hey proteins contain two domains, a basic domain and a helix-loop-helix domain. The DNA binding specificity is determined by the basic domain, while the helix-loop-helix domain allows for the formation of homodimers or heterodimers. Through interacting with the co-repressors or by blocking the transcriptional activators, both Hes and Hey proteins regulate gene transcription. These genes include transcription factors, c-Myc and NF-Kb2, cell cycle regulators, p21 and cyclin D1, growth factor receptors, such as HER2, and regulators of angiogenesis and apoptosis. (29)

Hypothesis

The Notch pathway acts as a tumor suppressor in the endometrium, with higher expression of pathway members in normal endometrium but decreased expression in endometrial cancer.

Specific Aims

1. Examine the qRT-PCR expression of Notch pathway members, including *HES1*, *HEYL*, *Notch4*, and *Jagged1*, in normal endometrium and various types of endometrial carcinoma.
2. Correlate Notch pathway gene expression with relevant clinical and pathological features of endometrial carcinoma, such as FIGO grade, stage of the tumor, and endometrioid and non-endometrioid histotype.

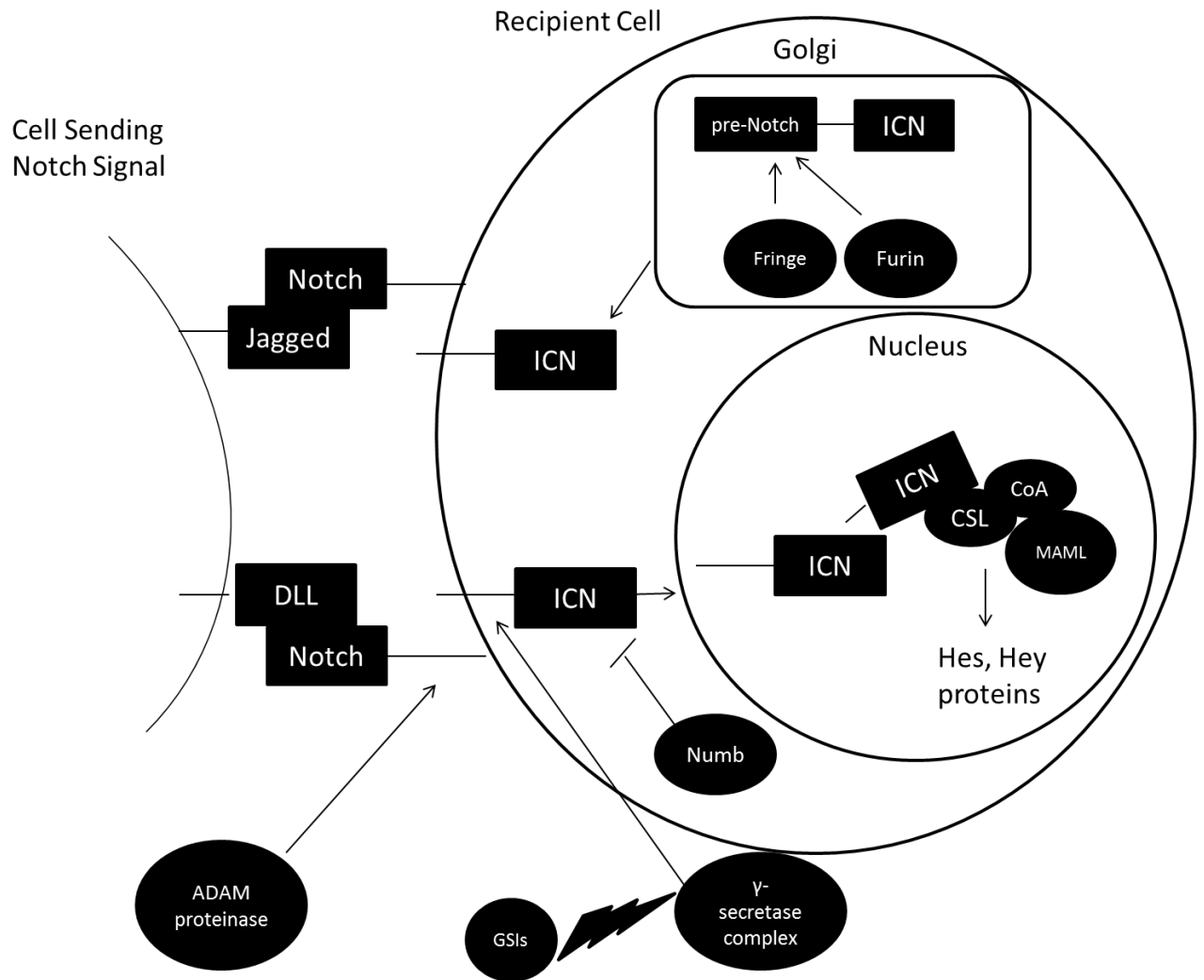


Figure 133. Notch Signaling Pathway. Notch ligands bind to the extracellular domain of the Notch receptors, causing the ligand-receptor complex to be activated and undergo several proteolytic cleavages. The activated form of Notch, Notch Intra-Cellular Domain (ICN) translocates to the nucleus, binds with transcription factors, and induces transcription of Notch target genes. ICN: Intra-cellular Notch; CoA: co-activators; GSI: γ -secretase inhibitor

Chapter 5

Notch Pathway Results

In order to determine which of the Notch pathway components play a significant role in the endometrium, we designed a Roche Notch-centric PCR array. See Chapter 2, Table 4, page 19 for a summary of the Notch pathway components that were represented on this array. PCR arrays combine the gene expression profiling feature of microarrays and the quantitative nature of real-time PCR. Additionally, PCR arrays can focus on a specific pathway, profiling the expression of all genes relevant to that pathway.

Using a subset of normal ($n = 9$) and endometrial cancer ($n = 63$) samples, we used the Notch-centric PCR array to analyze gene expression levels in four relevant categories: normal endometrium versus grade 1 EECs, normal endometrium versus grade 2 EECs, normal endometrium versus grade 3 EECs, and normal endometrium versus NEECs. Tables 7-10 summarize the results of these analyses. Of all the Notch components analyzed, *Notch4* and *Jagged1* consistently showed a significant difference in expression when comparing normal endometrium to the various grades of endometrial carcinomas represented in the array. As a result, we designed and performed qRT-PCR assays specifically for *Notch4* and *Jagged1* and examined their expression in the full set of normal endometrial tissues and endometrial carcinomas (figures 134-159).

Table 7. Roche Notch-centric PCR Array Gene Expression Levels: Normal Endometrium vs. Grade 1 EECs.

Gene	Fold Change	Difference (log scale)	p-value
<i>Notch1</i>	-1.093	-0.128	0.725
<i>Notch2</i>	-1.409	-0.495	0.234
<i>Notch3</i>	1.264	0.338	0.246
<i>Notch4</i>	-2.696	-1.431	0.031
<i>Jag1</i>	-1.124	-0.169	0.720
<i>Jag2</i>	1.167	0.223	0.707
<i>DLL1</i>	-2.086	-1.061	0.100
<i>DTX1</i>	-1.625	-0.701	0.574
<i>ADAM10</i>	-1.212	-0.278	0.341
<i>PSEN1</i>	-1.174	-0.231	0.360
<i>PSEN2</i>	1.120	0.164	0.680
<i>PSENEN</i>	1.556	0.638	0.112
<i>TFRC</i>	1.443	0.529	0.203
<i>TBP</i>	-1.356	-0.440	0.240

Table 8. Roche Notch-centric PCR Array Gene Expression Levels: Normal Endometrium vs. Grade 2 EECs.

Gene	Fold Change	Difference (log scale)	p-value
<i>Notch1</i>	1.139	0.188	0.727
<i>Notch2</i>	-1.163	-0.218	0.450
<i>Notch3</i>	1.209	0.273	0.569
<i>Notch4</i>	-2.728	-1.448	0.031
<i>Jag1</i>	-1.710	-0.774	0.031
<i>Jag2</i>	1.407	0.493	0.508
<i>DLL1</i>	-2.101	-1.071	0.110
<i>DTX1</i>	-1.451	-0.537	0.514
<i>ADAM10</i>	1.356	0.439	0.146
<i>PSEN1</i>	1.115	0.158	0.644
<i>PSEN2</i>	1.393	0.479	0.353
<i>PSENEN</i>	-1.001	-0.001	>0.99
<i>TFRC</i>	1.768	0.823	0.001
<i>TBP</i>	1.182	0.241	0.533

Table 9. Roche Notch-centric PCR Array Gene Expression Levels: Normal Endometrium vs. Grade 3 EECs.

Gene	Fold Change	Difference (log scale)	p-value
<i>Notch1</i>	-2.554	-1.353	0.007
<i>Notch2</i>	-2.144	-1.100	0.009
<i>Notch3</i>	-1.377	-0.461	0.306
<i>Notch4</i>	-3.040	-1.604	0.004
<i>Jag1</i>	-2.936	-1.554	0.0003
<i>Jag2</i>	1.492	0.577	0.242
<i>DLL1</i>	-2.387	-1.255	0.027
<i>DTX1</i>	-3.111	-1.637	0.062
<i>ADAM10</i>	-1.480	-0.565	0.137
<i>PSEN1</i>	-1.381	-0.465	0.116
<i>PSEN2</i>	1.102	0.141	0.765
<i>PSENEN</i>	-1.695	-0.761	0.105
<i>TFRC</i>	1.526	0.610	0.020
<i>TBP</i>	-1.743	-0.801	0.086

Table 10. Roche Notch-centric PCR Array Gene Expression Levels: Normal Endometrium vs. Non-Endometrioid Carcinomas.

Gene	Fold Change	Difference (log scale)	p-value
<i>Notch1</i>	-2.020	-1.015	0.114
<i>Notch2</i>	-1.059	-0.082	0.871
<i>Notch3</i>	1.108	0.148	0.676
<i>Notch4</i>	-2.638	-1.400	0.035
<i>Jag1</i>	-4.026	-2.009	0.002
<i>Jag2</i>	1.446	0.532	0.257
<i>DLL1</i>	-2.156	-1.109	0.049
<i>DTX1</i>	-1.979	-0.985	0.213
<i>ADAM10</i>	-1.865	-0.899	0.053
<i>PSEN1</i>	-1.504	-0.589	0.095
<i>PSEN2</i>	1.104	0.143	0.803
<i>PSENEN</i>	-1.285	-0.361	0.288
<i>TFRC</i>	1.261	0.335	0.440
<i>TBP</i>	-1.052	-0.073	0.862

Notch4

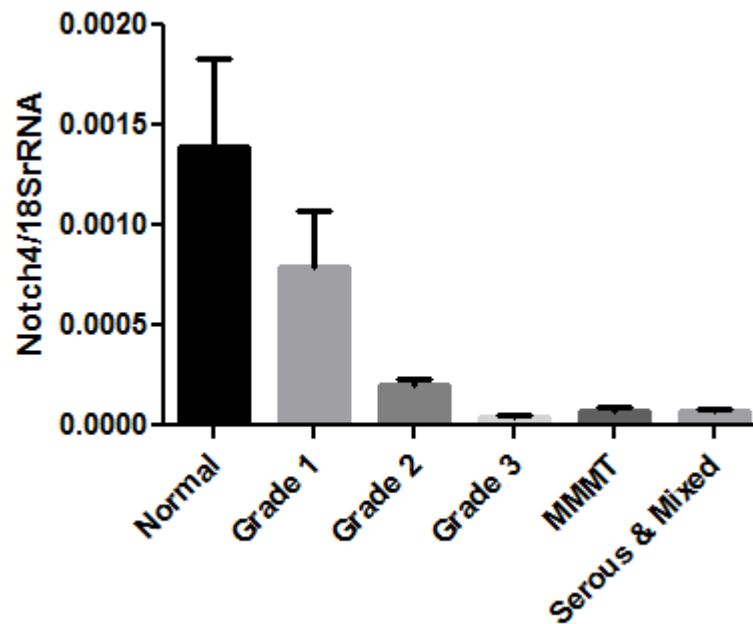


Figure 134. Expression of *Notch4* in normal endometrium and in endometrial carcinomas. This graph compares *Notch4* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of *Notch4* was decreased in endometrial carcinomas compared to normal.

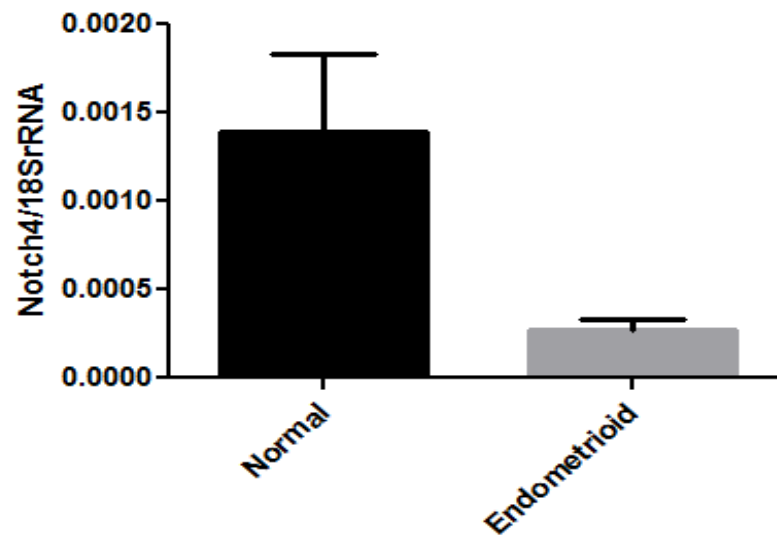


Figure 135. Expression of *Notch4* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall (p = <0.0001).

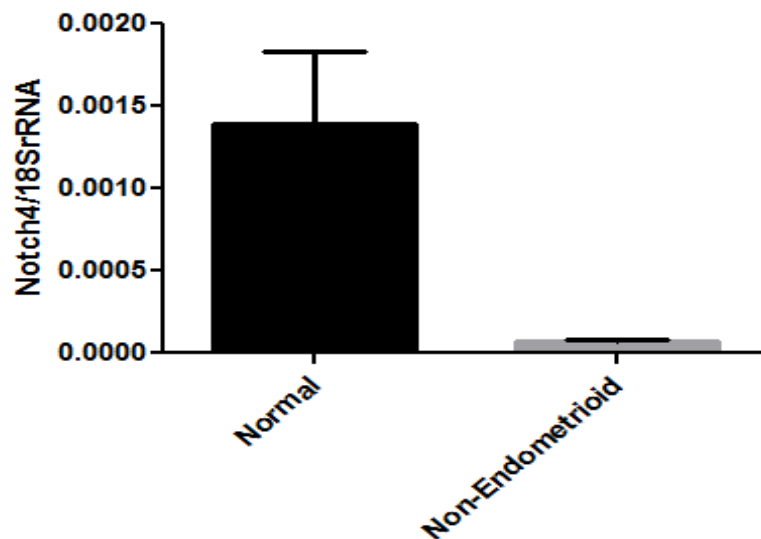


Figure 136. Expression of *Notch4* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = <0.0001).

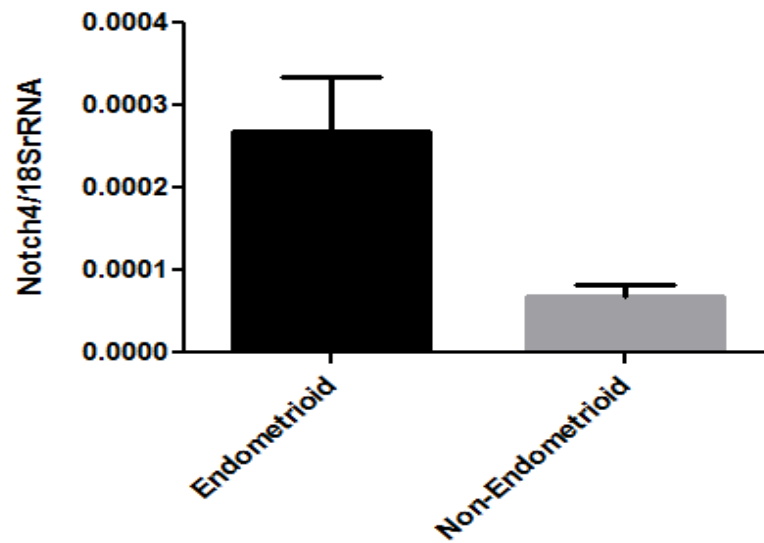


Figure 137. Expression of *Notch4* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0228).

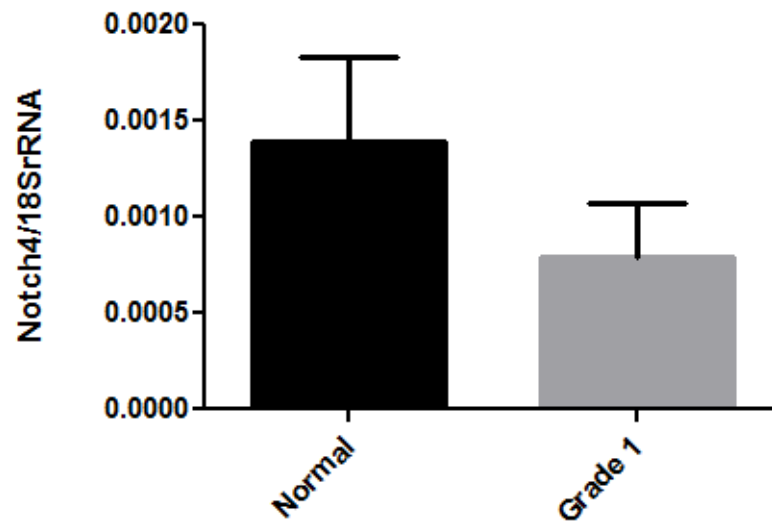


Figure 138. Expression of *Notch4* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Normal endometrium and grade 1 tumors have comparable expression (p = 0.2838).

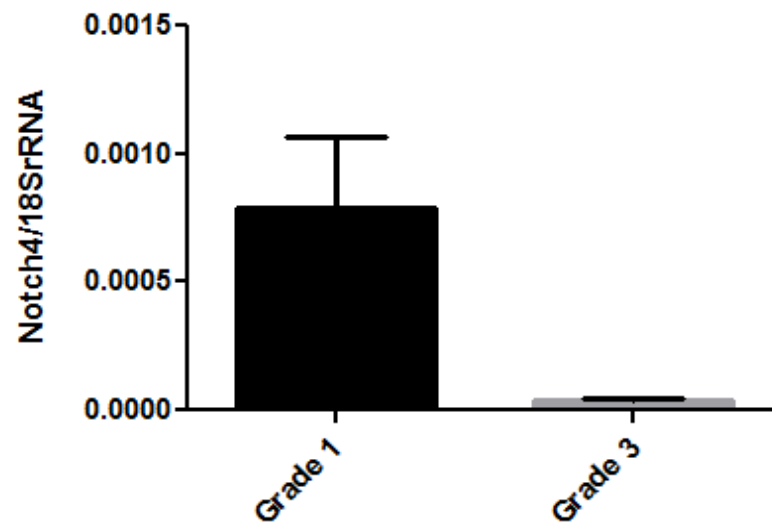


Figure 139. Expression of *Notch4* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 have significantly lower expression than grade 1 tumors (p = 0.0023).

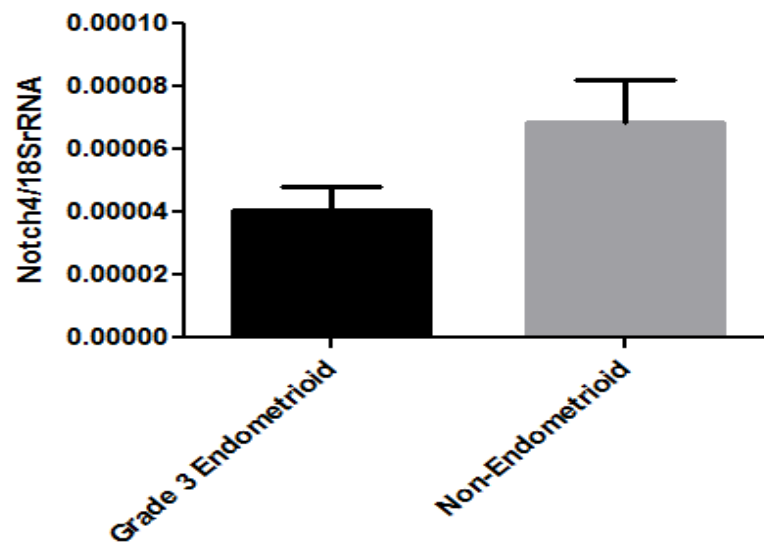


Figure 140. Expression of *Notch4* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significant (p = 0.1781).

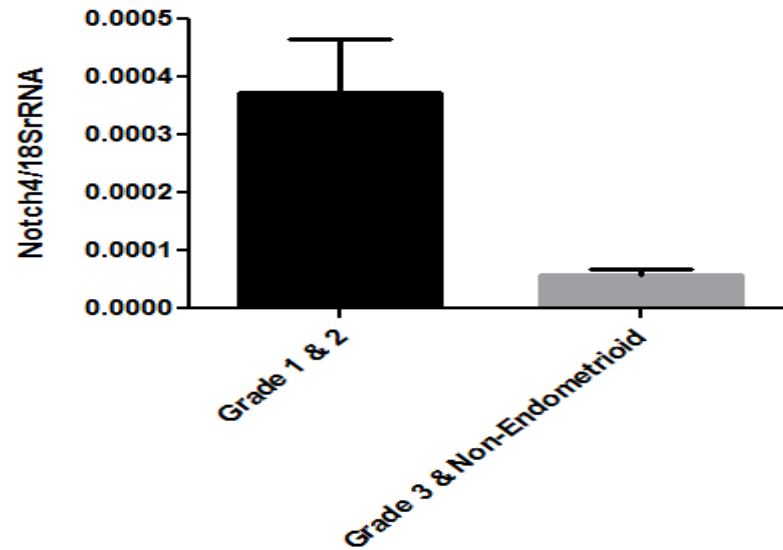


Figure 141. Expression of *Notch4* in grades 1 & 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = 0.0002).

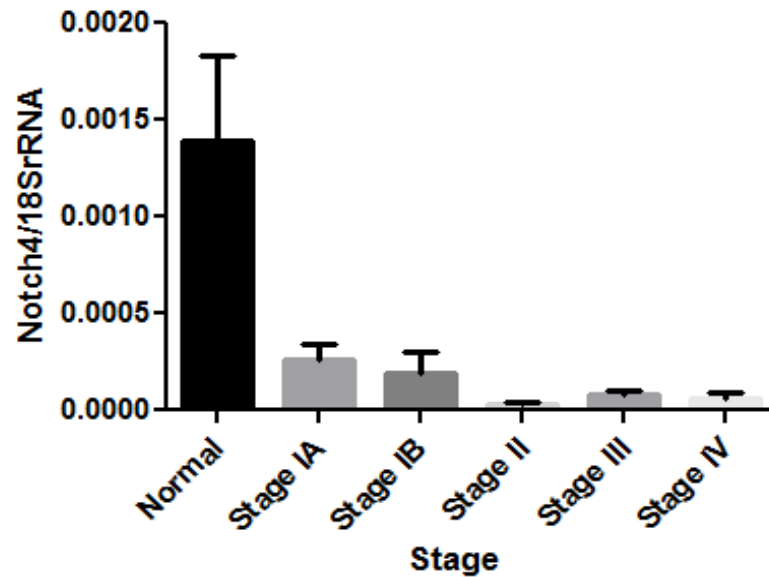


Figure 142. Expression of *Notch4* in normal endometrium and in endometrial carcinomas by FIGO stage.

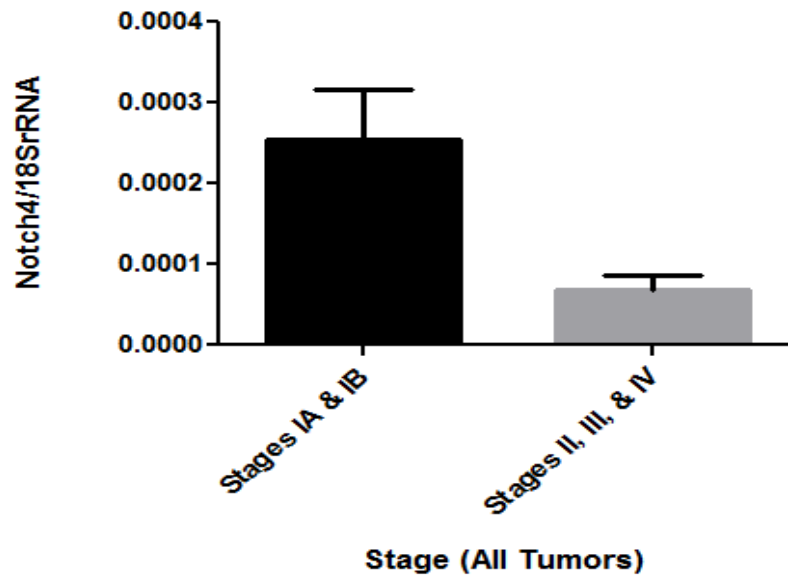


Figure 143. Expression of *Notch4* in stages IA & IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression (p = 0.0409).

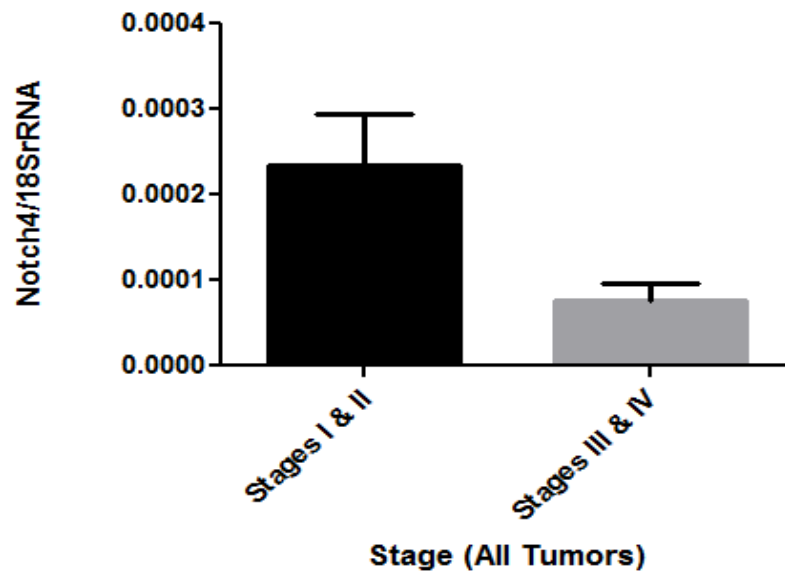


Figure 144. Expression of *Notch4* in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas. Higher stages have slightly lower expression, but this difference is not significant (p = 0.0970).

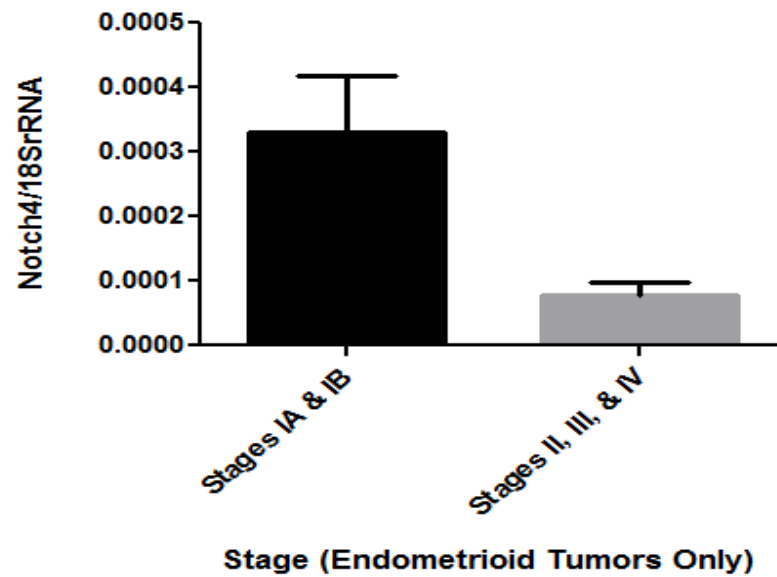


Figure 145. Expression of *Notch4* in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined. No significant difference is noted ($p = 0.0901$).

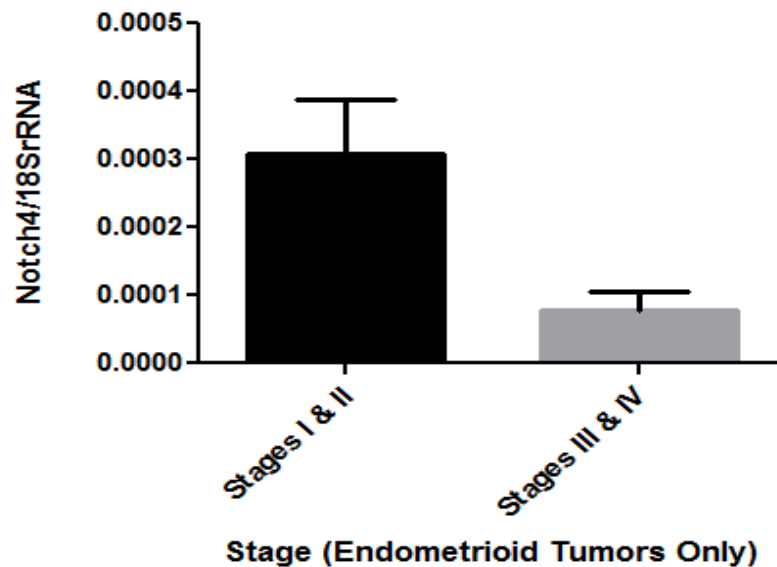


Figure 146. Expression of *Notch4* in stages I & II endometrioid endometrial carcinomas compared to higher stages. Higher stages have lower expression but this difference is not significant ($p = 0.1738$).

Our results found *Notch4* expression to be significantly different when comparing several endometrial tumor subtypes. *Notch4* expression was significantly higher in normal endometrial tissues compared to endometrial carcinomas ($p = <0.0001$). Higher expression was also seen in EEC samples compared to NEEC samples ($p = 0.0228$).

Jagged1

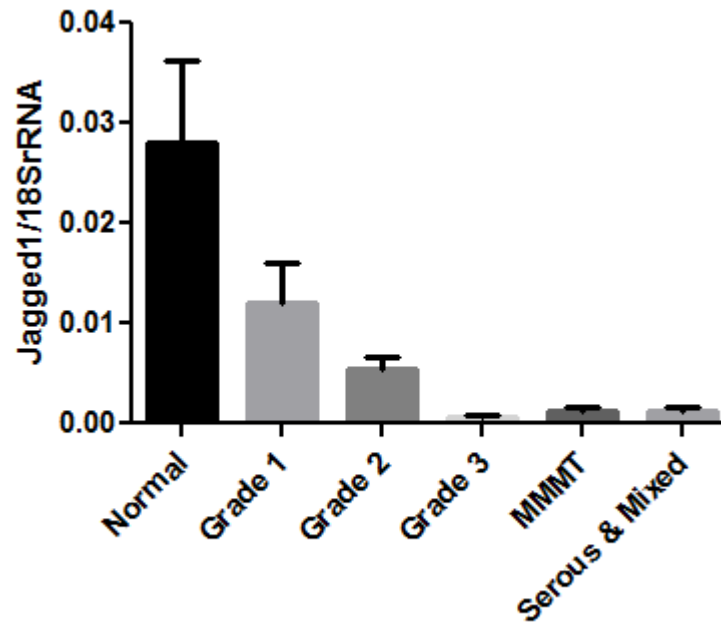


Figure 147. Expression of *Jagged1* in normal endometrium and in endometrial carcinomas. This graph compares *Jagged1* expression levels in normal endometrium (n = 10), endometrioid endometrial carcinomas (EECs), which includes grade 1 (n = 9), 2 (n = 22), and 3 (n = 18), and non-endometrioid endometrial carcinomas (NEECs), which includes malignant mixed mullerian tumors (MMMT; n = 14) and serous and mixed-serous endometrioid carcinomas (n = 15). Expression of *Jagged1* was decreased in endometrial carcinomas compared to normal.

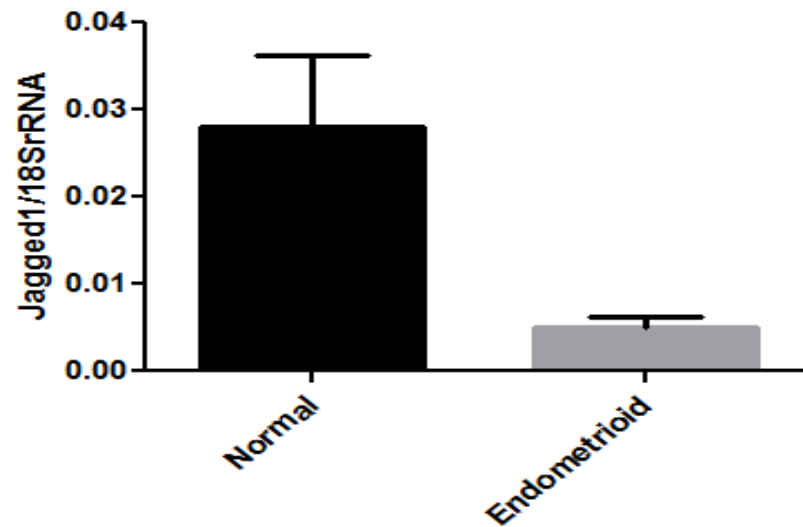


Figure 148. Expression of *Jagged1* in normal endometrium (n = 10) and in endometrioid endometrial carcinomas (grades 1, 2, and 3; total n = 49). Expression is significantly lower in the endometrioid tumors overall ($p = <0.0001$).

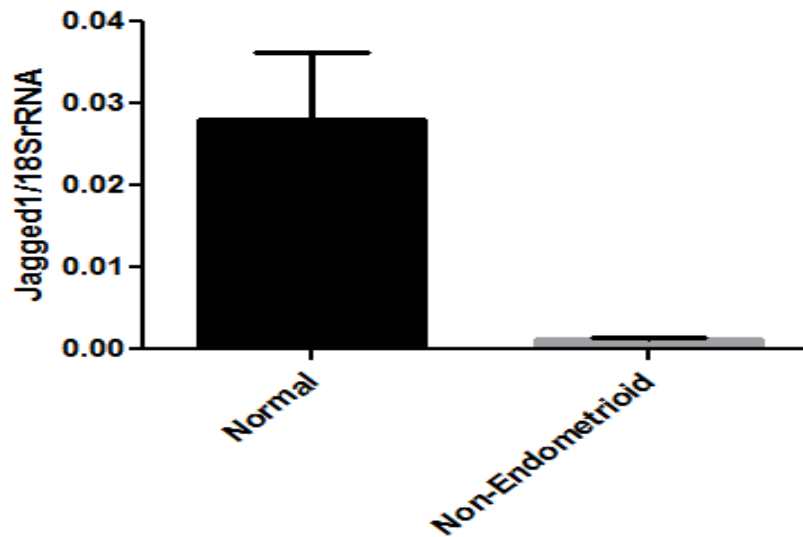


Figure 149. Expression of *Jagged1* in normal endometrium (n = 10) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression ($p = <0.0001$).

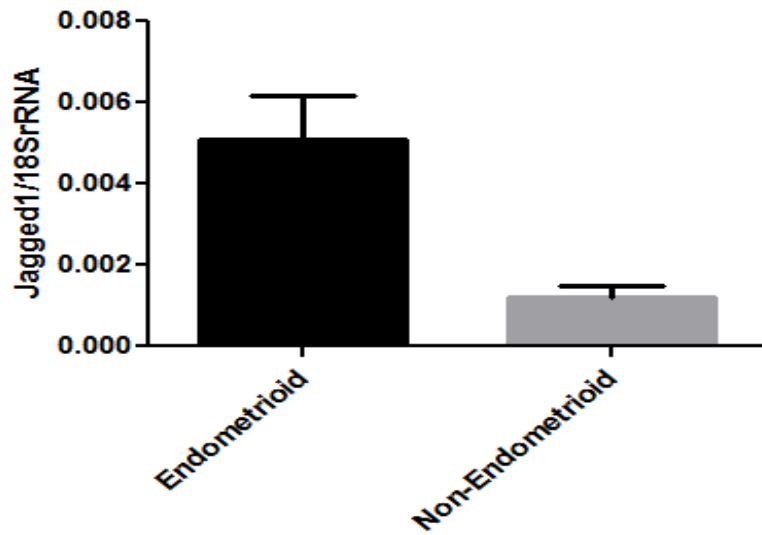


Figure 150. Expression of *Jagged1* in endometrioid endometrial carcinomas (grades 1, 2, 3; total n = 49) and in non-endometrioid endometrial carcinomas (n = 29). Non-endometrioid tumors have significantly lower expression (p = 0.0087).

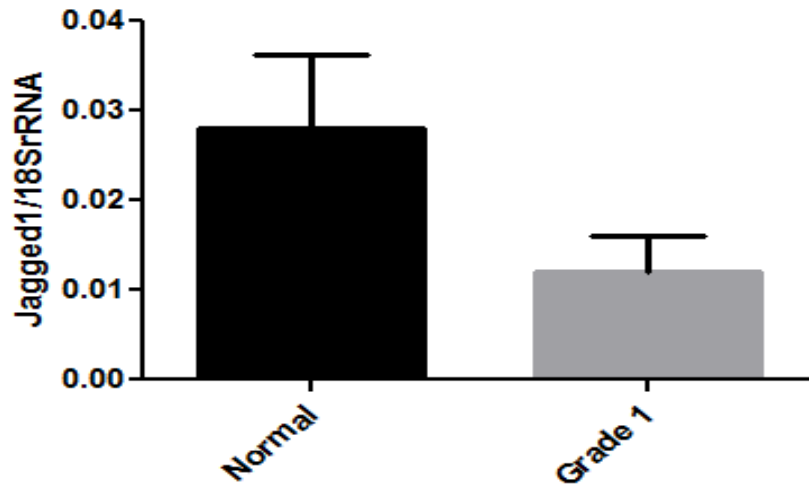


Figure 151. Expression of *Jagged1* in normal endometrium (n = 10) and in grade 1 endometrioid endometrial carcinomas (n = 9). Normal endometrium and grade 1 tumors have comparable expression (p = 0.1040).

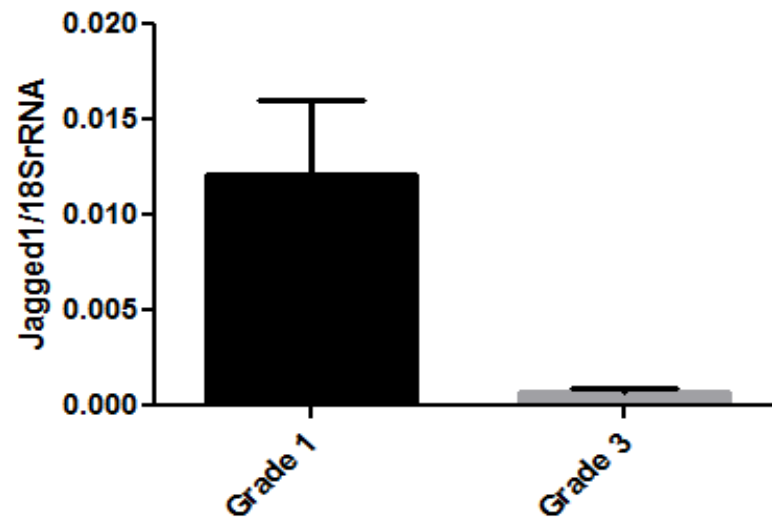


Figure 152. Expression of *Jagged1* in grade 1 EECs (n = 9) and in grade 3 EECs (n = 18). Grade 3 tumors have significantly lower expression compared to grade 1 tumors (p = 0.0006).

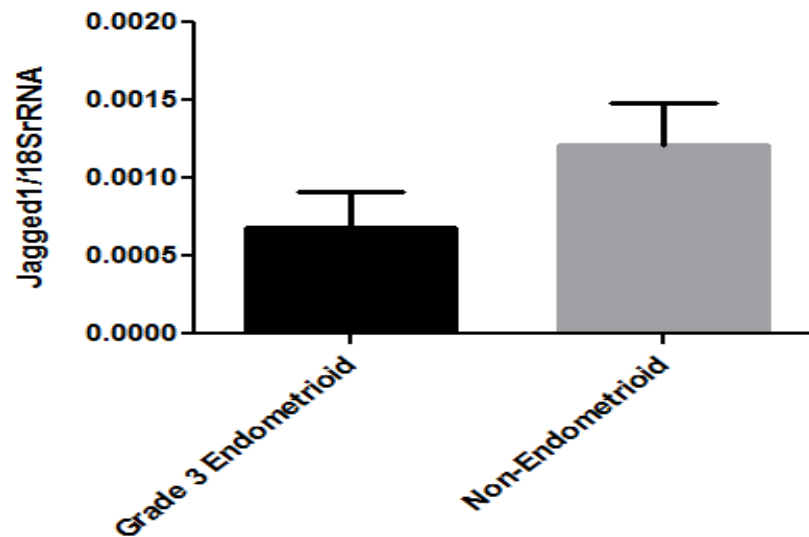


Figure 153. Expression of *Jagged1* in grade 3 EEC samples (n = 18) and in NEEC samples (n = 29). Expression in grade 3 and non-endometrioid tumors is not significant (p = 0.1798).

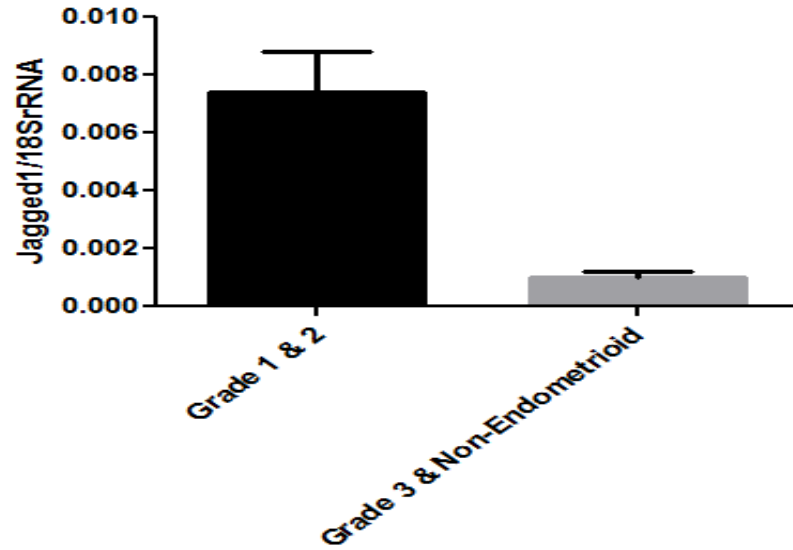


Figure 154. Expression of *Jagged1* in grades 1 & 2 EECs (n = 31) and in grade 3 EECs and NEECs (n = 47). The grade 3 and non-endometrioid tumors have significantly lower expression compared to the grades 1 and 2 group (p = <0.0001).

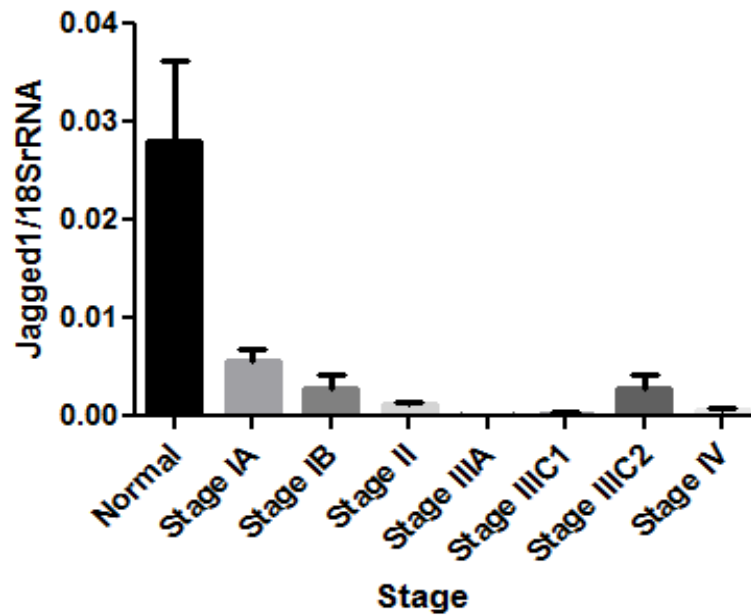


Figure 155. Expression of *Jagged1* in normal endometrium and in endometrial carcinomas by FIGO stage.

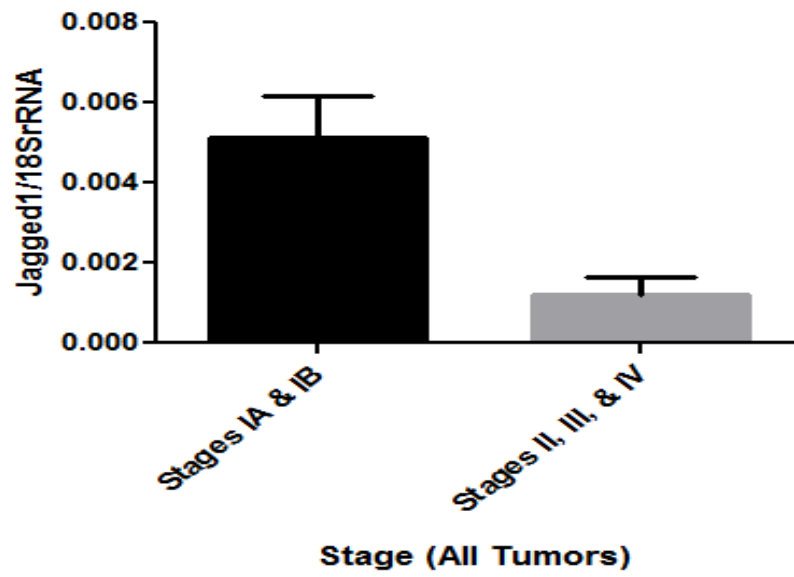


Figure 156. Expression of *Jagged1* in stages IA & IB endometrial carcinomas (n = 48) compared to combined stages II, III, and IV (n = 30). Higher stages have significantly lower expression (p = 0.0082).

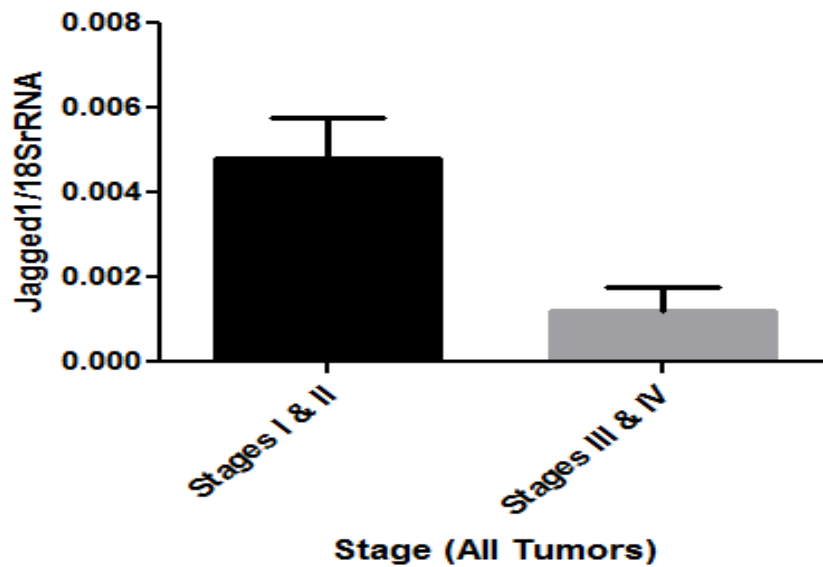


Figure 157. Expression of *Jagged1* in stage I & II endometrial carcinomas and in stages III & IV endometrial carcinomas. Higher stages have significantly lower expression (p = 0.0208).

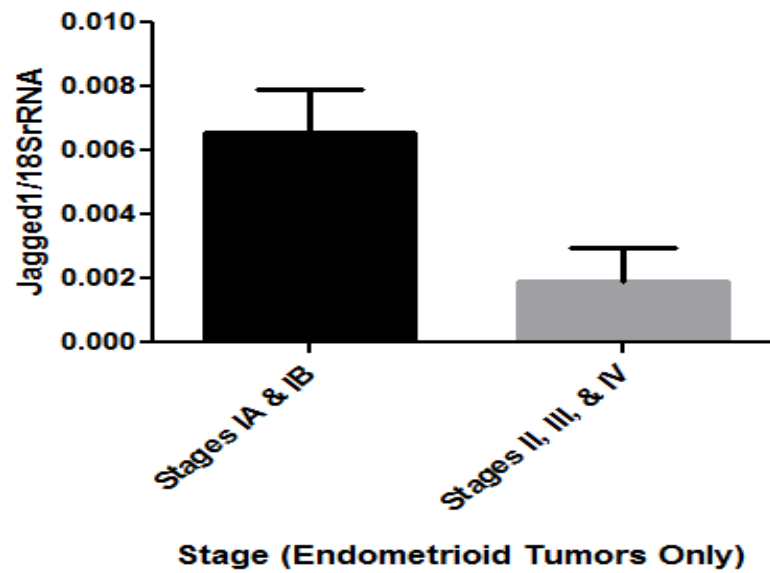


Figure 158. Expression of *Jagged1* in stages IA & IB endometrioid endometrial carcinomas compared to higher stages combined. Higher stages have significantly lower expression ($p = 0.0415$).

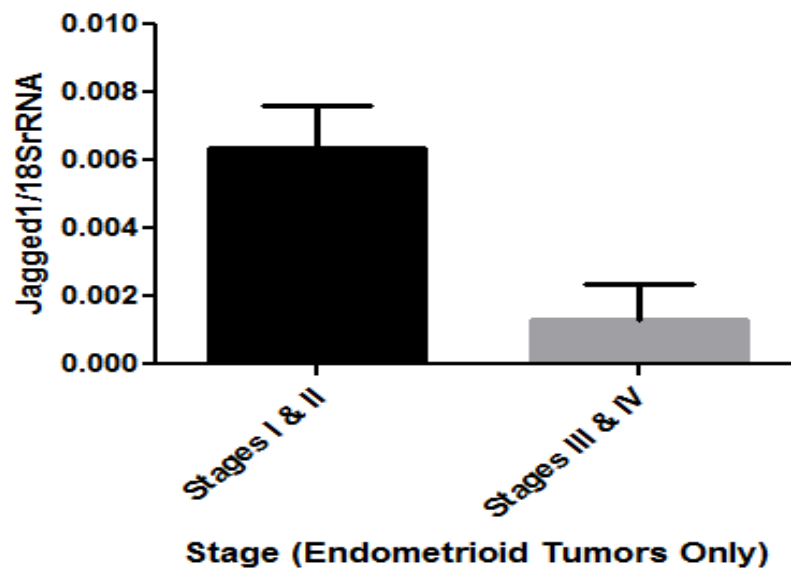


Figure 159. Expression of *Jagged1* in stages I & II endometrioid endometrial carcinomas compared to higher stages. Higher stages have significantly lower expression ($p = 0.0392$).

Our results found *Jagged1* expression to be significantly different when comparing several endometrial tumor subtypes. *Jagged1* expression was significantly higher in normal endometrial tissues compared to endometrial carcinomas ($p = <0.0001$). *Jagged1* expression was significantly lower in poorly differentiated endometrial carcinomas compared to low grade, well-differentiated endometrial carcinomas.

Summary

Our results show a significant decrease in expression level of both *Notch4* and *Jagged1* in correlation with higher FIGO grade and stage. This is comparable to the expression levels of both target genes of the Notch signaling pathway, *HEYL* and *HES1*. Table 11 provides a summary of the qRT-PCR results for these four components of the Notch pathway. All four components were significantly down-regulated in poorly differentiated, clinically invasive endometrial carcinomas compared to normal endometrium and well-differentiated, low grade endometrial carcinomas. Based on this data, we believe the Notch pathway to play a tumor suppressive role in endometrial cancer.

Table 11. Summary of qRT-PCR Results: Notch Pathway Components.

	Normal vs. Grade 1 Endometrioid	Endometrioid vs. Non- endometrioid	Grade 1 Endometrioid vs. Grade 3 Endometrioid	Stages I and II vs. Stages III and IV (Endometrioid tumors)
<i>HEYL</i>	X		X	
<i>HES1</i>	X	X	X	X
<i>Notch4</i>		X	X	
<i>Jagged1</i>		X	X	X

Chapter 6

Discussion

The most common gynecologic malignancy and the fourth most common cancer among women is endometrial carcinoma (1, 2). As with other cancer types, endometrial carcinoma has been the focus of numerous studies to characterize its molecular pathogenesis, including the large-scale efforts of The Cancer Genome Atlas. Despite these efforts, the molecular events affiliated with the progression of endometrial carcinoma from normal endometrium are still largely uncharacterized. Therefore, the identification of molecular markers of tumor development would have a significant clinical impact. The purpose of this thesis was to determine gene expression levels associated with endometrial cancer development and identify molecular markers that could potentially be used for therapeutic targeting or as biomarkers of cancer risk in high risk populations, such as obese women or women with Lynch Syndrome.

Many microarray studies have been performed to identify genes that are abnormally expressed in endometrial tumors compared to normal endometrium (12). Based on a previous microarray study performed on normal endometrium from obese women and cancerous endometrium from thin and obese women, we validated a number of genes which were significantly up-regulated or down-regulated. Furthermore, two of the ten genes studied belong to the same signaling pathway, the Notch pathway. While the role of various Notch pathway members has been documented in other cancer types, their specific function in endometrial cancer has not been determined yet. The Notch pathway has been reported to function in cell differentiation, proliferation, apoptosis, as well as vascular development and

angiogenesis (32). Based on our observation of low expression of *HEYL* and *HES1*, target genes of the Notch pathway, we hypothesized this pathway to have a tumor suppressive role in endometrial cancer. Following validation of the initial 10 genes, this thesis then primarily focused on a comprehensive examination of Notch pathway components, including ligands, receptors, processing enzymes, and downstream indicators of pathway activation, in normal endometrium, endometrioid adenocarcinoma, and non-endometrioid adenocarcinoma.

First 10 genes

Several of the genes initially validated gave promising results and could be targeted for therapeutic implications. We compared gene expression levels according to endometrial cancer grade and stage and found some genes to show a dramatic difference in expression depending on the tumor histotype. We primarily focused on four comparisons: normal endometrium versus grade 1 endometrioid tumors; endometrioid tumors versus non-endometrioid tumors; grade 1 endometrioid carcinomas versus grade 3 endometrioid carcinomas; and stages I and II endometrioid endometrial carcinomas versus stages III and IV endometrioid endometrial carcinomas. Based on the results of the qRT-PCR, we categorized the ten genes into one or more of these four comparison groups (see Table 6).

OLFM1, *SPRY1*, *PEG3*, *EDNRA*, and *HEYL* had high expression levels in normal endometrium, but were significantly down-regulated in grade 1 endometrioid tumors. The low expression levels in endometrial cancer suggest that these genes may normally function as tumor suppressors and/or growth inhibitors that are somehow being inactivated or inhibited. Further studies are necessary to determine the mechanism responsible for this

action. Subsequently, therapies could be developed that target the mechanism causing the genes to lose their tumor suppressive function.

Various genes have already been identified to help distinguish endometrioid from non-endometrioid tumors. For example, *EIG121*, a gene induced by exogenous and endogenous estrogens, is highly elevated in complex atypical hyperplasia and grade 1 endometrioid carcinomas, but not in non-endometrioid carcinomas, such as MMMT (33). Similarly, we found *P-cadherin* and *VAV3* had higher expression levels in endometrioid endometrial carcinomas but had a significant decrease in expression in non-endometrioid endometrial carcinomas. As a result, these genes would also be especially useful in distinguishing endometrioid from non-endometrioid tumors.

When comparing grade 1 endometrioid tumors to grade 3 endometrioid type tumors, *PIK3R1*, *EFNA1*, *EDNRA*, and *HES1* showed substantially higher expression levels in grade 1 endometrioid type tumors. Interestingly, most of these genes were not helpful in distinguishing low stage (I and II) from higher stage (III and IV) endometrial carcinomas. Only *HES1* showed higher expression levels in low stage endometrial carcinomas and significantly lower expression levels in high stage endometrial carcinomas.

As previously stated, several of the genes validated could potentially be used to help distinguish normal endometrium from grade 1 endometrioid adenocarcinoma, as well as low grade endometrial carcinomas from high grade endometrial carcinomas. It has been observed that for many genes and pathways, there is little difference between normal endometrium, complex atypical hyperplasia and grade 1 endometrioid tumors (34, 35). Therefore, it can be difficult to distinguish between the three. Also, grade 1 endometrioid tumors are well-differentiated, while grade 3 endometrioid tumors are poorly-differentiated

and clinically invasive. Gene panels that target these two comparisons would be extremely helpful to identify the subset of women who are at-risk for endometrial cancer development or for metastasis.

Oncotype Dx is a qRT-PCR gene panel used to help guide the therapy of women with ER positive, lymph node negative -breast cancer. It can be run on formalin-fixed, paraffin-embedded tissues. This panel helps to identify the subset of women who might be at highest risk for recurrence/metastasis. These women would then be treated with chemotherapy in addition to tamoxifen/aromatase inhibitors (36). Using the genes that had a distinct difference in expression between normal endometrium and grade 1 EEC, a similar type of qRT-PCR gene panel could be developed in order to identify the subset of women who might be at highest risk for endometrial cancer development. qRT-PCR could be performed on formalin-fixed, paraffin-embedded benign endometrial biopsies to determine the expression levels of *OLFMI*, *EFNA1*, *EDNRA*, *HES1*, *SPRY1*, and *PIK3R1*. The women whose tissues have low expression levels of these genes could begin chemoprevention counter measures, begin a weight loss program if obese, or consider prophylactic hysterectomy to avoid endometrial cancer development. Additionally, another gene panel which screens for the genes which have significantly low expression levels in poorly differentiated endometrial carcinomas would help to identify women who may be at highest risk for metastasis. In this case, women could receive adjuvant treatment following hysterectomy to help prevent recurrence or metastasis.

Heterogeneity in Cancer

Cancer is a heterogeneous disease – pathways active in one cancer type may not be active at all in a different type. As a result, the effect or impact of a specific gene may be different depending on the specific cancer type. For a number of the genes validated in our study, the results in endometrial cancer are very different from what has been reported for other cancer types.

VAV3 is over-expressed in breast and prostate cancers in correlation with poorly differentiated tumors (13). However, our study found *VAV3* expression to be higher in well-differentiated endometrial carcinomas and down-regulated in poorly differentiated endometrial carcinomas. Increased expression of *EFNA1* contributes to tumorigenesis in several different cancer types, such as melanoma, bladder, gastric, cervical and ovarian cancers (21). However, we found *EFNA1* expression to be down-regulated in correlation with poorly differentiated endometrial carcinomas. *EDNRA* and *HES1* over-expression is also correlated with high grade ovarian carcinomas (23, 27), but the opposite is seen in high grade endometrial carcinomas. Additionally, *HEYL* expression is higher in invasive breast cancers (25), but we found *HEYL* to be down-regulated in the more clinically invasive and poorly differentiated endometrial carcinomas. Based on these results, the extent of heterogeneity in cancer is apparent.

Studies have reported that almost 90% of signaling pathways are different in cells of various cancers (37). What causes a pathway to be active in one cancer type but not active in another type? While there is no definite explanation, many possibilities have been suggested. The outcome could be dependent on the specific cell type, chronology of the mutation, or that the intermediate proteins of a signaling pathway are different from one

cancer type to another. Regardless, it is important to keep in mind that cancers can be heterogeneous and develop in a different manner. Drugs or therapies targeted for one cancer type therefore may not have the same successful outcome on another cancer type.

Notch Role in Cancer

Some signaling pathways have been reported to have both tumor suppressive and tumor promoting roles. For example, TGF- β can act as an oncogene or as a tumor suppressor in a context dependent manner. Typically, early in tumorigenesis, TGF- β acts as a tumor suppressor, functioning to inhibit proliferation, induct apoptosis, activate autophagy, induct senescence, inhibit motility and promote differentiation (38). However, as tumors progress, epigenetic and genetic changes to the tumor cell genome cause TGF- β to switch from a tumor suppressor to a tumor promoter. These epigenetic and genetic changes include loss of Smad4, overexpression of Six1, down-regulation of DAB2, and *p53* mutation. As a result, later in tumorigenesis, TGF- β promotes proliferation, epithelial to mesenchymal transition, metastasis, and cell motility (38). Inactivation of the vital TGF- β pathway members, such as TGF- β receptors, is also responsible for disabling the tumor suppressive effect of TGF- β . This occurs in colorectal, pancreatic, ovarian, and gastric cancers (39).

The Notch signaling pathway also has both oncogenic and tumor suppressive roles in various cancers. There is much debate as to why Notch acts differently in different tissues. Because p21 induction typically inhibits cell growth, one possible explanation is that its role is dependent on the status of the G1 cell cycle checkpoint. p21 is responsible for the formation of the cyclinD/CDK4 complex, but it does not inactivate the complex. However, p21 does inactivate the CDK2/cyclin E complex and is also responsible for CDK4-

dependent Rb phosphorylation. Therefore, the expression levels of p21, cyclin D1, cyclin E, and CDKs 4, 5, and 2 determine whether Notch activation causes cell growth or growth arrest. For example, if the G1 checkpoint is inactivated by loss of p16 or up-regulation of D cyclins, Notch-1 mediated induction of p21 may not inhibit cell growth. (40)

Notch as an oncogene

Notch involvement in cancer was first seen in T-acute lymphoblastic leukemia/lymphoma (T-ALL) in 1991. A translocation was found in T-ALL patients, which caused the fusion of the 3' region of *Notch1* into the TCR β locus and led to overexpression of the activated form of Notch1. The translocation seemed to be rare, but several years later, activating *Notch1* mutations were seen in about 56% of T-ALL patients. (30, 31)

After this discovery, Notch signaling was linked to several solid tumors, including breast cancer, medulloblastoma, colorectal cancer, non-small cell lung cancer, and melanomas (31). Notch's oncogenic potential was first seen in mouse mammary tumor virus (MMTV) driven breast cancer. Studies have shown that incorporating MMTV in specific loci caused dysregulated expression of adjacent genes and growth of tumorigenic clones. Loci characterization revealed a constitutively active form of Notch4. Therefore, Notch activation evidently causes mammary tumors in mouse models and in human breast cancer, *Notch* or *Jag1* up-regulation has been reported to correlate with poor prognosis (31).

The expression of Notch receptors and their target genes is also up-regulated in primary human melanomas, in which constitutively active Notch1 promotes melanoma progression. The oncogenic role of Notch in melanoma cells correlates with the activation of

Wnt signaling. Increased Notch activity in melanoma cells has also been correlated with activation of the PI3K/Akt signaling pathway. (30)

Notch as a tumor suppressor

Studies have shown the tumor suppressive functions of Notch to occur through several mechanisms. First, it seems that one Notch target gene, p21, contributes to its tumor suppressive effects through inhibiting Wnt4a expressions and Wnt signaling in normal epithelium. It is also possible there is negative cross talk between Notch and p63, leading to deregulation of the balance between differentiation and self-renewal. (30)

The conditional deletion of Notch1 results in an increase of the basal epidermal layer in the skin. Notch1 loss of function has also been reported to result in spontaneous basal cell carcinomas in older mice. Additionally, the combined deletion of Notch1 and Notch2 or the conditional Notch loss-of-function through the deletion of Nicastrin, a vital component of the gamma-secretase complex, results in a myeloproliferative syndrome with common features of chronic myelomonocytic leukemia. (30)

Studies have also reported Notch to act as a tumor suppressor in head and neck squamous cell carcinomas (HNSCC). Out of 21/120 patients analyzed, Agrawal et al. (41) identified 28 different *Notch1* mutations, 11 of which were insertion/deletions or nonsense mutations resulting in Notch loss-of-function. Another study by Stransky et al. (42) identified *Notch1* mutations in 11% of HNSCC patients analyzed, with an additional 11% of patients having *Notch2* or *Notch3* mutations. The mutations in this study were missense, nonsense, or insertion/deletions which targeted the extracellular domain of the Notch receptors, leading to loss-of-function. Furthermore, Notch suppresses growth and induces

apoptosis in B cell tumors, providing additional evidence of tumor suppressive functions of the Notch pathway. (31)

Notch and Endometrial Cancer

The human endometrium undergoes tissue remodeling due to hormonal changes during every reproductive cycle. As a result, angiogenesis, the formation of new blood vessels, contributes to the remodeling of the endometrium. Deregulation of angiogenesis and the factors that promote or inhibit it relates to histological differentiation and may predispose endometrial cells to become malignant. The Notch pathway has been reported to contribute to angiogenesis and tissue remodeling. (32)

Cobellis et al. (32) investigated the localization and pattern of expression of Notch components, Notch1, Notch4, and Jagged1, in physiological and pathological human endometrium. Through immunohistochemistry, they found both Notch1 and Notch4 to have moderate expression levels in the glandular epithelium during the proliferative phase but observed an increase in Notch1 expression and a decrease in Notch4 expression during the secretory phase. Jagged1 had low expression levels in both the proliferative and secretory phases of the physiological endometrium examined. In the pathological specimens examined through immunohistochemistry, there was an increase in expression of Notch1 from polyps to carcinomas while both Notch4 and Jagged1 decreased in expression. It should be noted that the immunohistochemical expression for Notch pathway members, even in the normal endometrium, was very faint in the photomicrographs presented. This calls into question the reliability of the antibodies used for this study. It is possible the immunohistochemistry is underestimating the expression of Notch pathway components in the normal endometrium.

The results reported by Cobellis et al. (32) on the decrease in expression levels of Notch4 and Jagged1 from polyps to endometrial carcinomas are similar to the results we found through qRT-PCR. Both of these results suggest that Notch4 and Jagged1 could function as tumor suppressor proteins in the human endometrium and deregulation of these functions could lead to cancer.

The Notch pathway has been reported to contribute to cell differentiation, angiogenesis and vascularization. Because the endometrium undergoes tissue remodeling during the reproductive cycle, it is important to effectively regulate the factors that contribute to this process. Inhibition or inactivation of the factors that are involved in cell differentiation and angiogenesis is a possible reason for tumor progression in endometrial cancer. Further studies are needed on the cross talk between Notch components and factors responsible for differentiation and angiogenesis in order to understand the complete mechanism for endometrial cancer development.

Clinical Implications of Targeting the Notch Pathway

Clinically useful agonists that activate the Notch pathway have not yet been described. However, there are a variety of genetic and pharmacological therapies that can inhibit Notch signaling at different levels of the pathway. Notch receptors and ligands can be inhibited by selective and non-selective strategies such as monoclonal antibodies, receptor decoys, soluble ligands, or inhibition of enzymes responsible for receptor cleavage, such as gamma secretase inhibitors or ADAM inhibitors. (29)

Gamma secretase inhibitors (GSIs) are the furthest in development as possible cancer treatment agents. GSIs function by inhibiting the gamma secretase complex, which normally

cleaves Notch into its active form. Through inhibiting this process, Notch remains inactive and further signaling is stopped. Merck and Roche have entered GSIs into clinical trials. In a phase I trial, GSI MK-0752 (Merck) was given to 7 patients with advanced solid tumors and 14 patients with advanced breast cancer. At all doses, MK-0752, inhibited gamma secretase and decreased Abeta40, a product of gamma secretase cleavage, by 46% (29). GSIs have also shown antitumor activity in several human cancer cell lines. They have reduced tumor growth and vasculature in glioblastoma and lung cancer cell lines, induced growth arrest in T-ALL cells, and induced apoptosis in melanoma cell lines. (29)

Limitations

Although the research has reached its aims, there were some limitations to this study. Due to the lack of commercially available antibodies for the Notch pathway components, we were unable to perform immunohistochemistry to test for the protein levels of Notch components in normal and endometrial cancer samples. Also, since we were unable to perform this technique, it was difficult to compare our results to published literature that employed immunohistochemistry to determine Notch function in endometrial cancer. One approach that could help in localization would be to design in situ hybridization or fluorescent in situ hybridization probes for some of the relevant Notch pathway components. Finally, the amount of normal endometrial tissue samples and grade 1 endometrial carcinomas tissue samples available for this study was low due to lack of patients admitted to MD Anderson Cancer Center with benign lesions or low grade tumors.

Chapter 7

References

1. Saghir, F.S.A, Rose, I.M., Dali, A.Z.H.M., Shamsuddin, Z., Jamal, A.R.A., and Mokhtar, N.M. 2010. Gene expression profiling and cancer-related pathways in type 1 endometrial carcinoma. *Int J Gynecol Cancer* 20: 724-731.
2. Bansal, N., Yendluri, V., and Wenham, R.M. 2009. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. *Cancer Control* 16: 8-13.
3. Siegel, R., Naishadham, D., and Jemal, A. 2012. Cancer Statistics. *CA: A Cancer Journal for Clinicians* 62: 10-29.
4. Mutter, G.L., Lin, M.C., Fitzgerald, J.T., Kum, J.B., Baak, J.P, Lees, J.A., Weng, L.P., and Eng, C. 2000. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst* 92: 924-930.
5. Lax, S.F., Kendall, B., Tashiro, H., Slebos, R.J., and Hendrick, L. 2000. The frequency of p53, K-RAS mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. *Cancer* 88: 814-824.
6. Modesitt, S.C. 2012. Missed opportunities for primary endometrial cancer prevention. *Obstetrics and Gynecology* 120: 989-991.
7. Bozzao, C., Lastella, P., and Stella, A. 2011. Anticipation in Lynch Syndrome: where we are where we go. *Current Genomics* 12: 451-465.

8. Fader, A.N., Arriba, L.N., Frasure, H.E., and von Gruenigen, V.E. 2009. Endometrial cancer and obesity: epidemiology, biomarkers, prevention, and survivorship. *Gynecologic Oncology* 114: 121-127.
9. Van Marck, V., Stove, C., Jacobs, K., Van den Eynden, G., and Bracke, M. 2011. P-Cadherin in adhesion and invasion: opposite roles in colon and bladder carcinoma. *Int. J. Cancer* 128: 1031-1044.
10. Stefansson, I.M., Salvesen, H.B., and Akslen, L.A. 2004. Prognostic impact of alterations in P-Cadherin expression and related cell adhesion markers in endometrial cancer. *Journal of Clinical Oncology* 22: 1242-1252.
11. Wu, L., Chang, W., Zhao, J., Yu, Y., Tan, X., Su, T., Zhao, L., Huang, S., Liu, S., and Cao, G. 2010. Development of autoantibody signatures as novel diagnostic biomarkers of non-small cell lung cancer. *Clin Cancer Res* 16: 3760-3768.
12. Wong, Y.F., Cheung, T.H., Lo, K.W.K., Yim, S.F., Siu, N.S.S., Chan, S.C.S., Ho, T.W.F., Wong, K.W.Y., Yu, M.Y., Wang, V.W. Li, C., Gardner, G.J., Bonome, T., Johnson, W.B., Smith, D.I., Chung, T.K.H., and Birrer, M.J. 2007. Identification of molecular markers and signaling pathway in endometrial cancer in Hong Kong Chinese women by genome-wide gene expression profiling. *Oncogene* 26: 1971-1982.
13. Lee, K., Liu, Y., Mo, J.Q., Zhang, J., Dong, Z., and Lu, S. 2008. Vav3 oncogene activates estrogen receptor and its overexpression may be involved in human breast cancer. *BMC Cancer* 8.

14. Mason, J.M., Morrison, D.J., Basson, M.A., and Licht, J.D. 2006. Sprouty proteins: multifaceted negative feedback regulators of receptor tyrosine kinase signaling. *TRENDS in Cell Biology* 16: 45-54.
15. Darimipourain, M., Wang, S., Ittmann, M., and Kwabi-Addo, B. 2011. Transcriptional and post-transcriptional regulation of Sprouty1, a receptor tyrosine kinase inhibitor in prostate cancer. *Prostate Cancer and Prostatic Diseases* 14: 279-285.
16. Fernandez, S.V., and Russo, J. 2010. Estrogen and xenoestrogens in breast cancer. *Toxicologic Pathology* 38: 110-122.
17. Liu, P., Cheng, H., Roberts, T.M., and Zhao, J.J. 2009. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nature Reviews: Drug Discovery* 8: 627-644.
18. Cheung, L.W.T., Hennessy, B.T., Li, J., Yu, S., Myers, A.P., Djordjevic, B., Lu, Y., Stemke-Hale, K., Dyer, M.D., Zhang, F., Ju, Z., Cantley, L.C., Scherer, S.E., Liang, H., Lu, K.H., Broaddus, R.R., and Mills, G.B. 2011. High frequency of *PIK3R1* and *PIK3R2* mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discovery* 1: 170-185.
19. Dowdy, S.C., Gostout, B.S., Shridhar, V., Wu, X., Smith, D.I., Podratz, K.C., and Jiang, S. 2005. Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. *Gynecologic Oncology* 99: 126-134.
20. Beauchamp, A. and Debinski, W. 2011. Ephs and ephrins in cancer: ephrin-A1 signaling. *Seminars in Cell & Developmental Biology* 23: 109-115.

21. McCarron, J.K., Stringer, B.W., Day, B.W., and Boyd, A.W. 2010. Ephrin expression and function in cancer. *Future Oncol.* 6: 165-176.
22. Merritt, W.M., Kamat, A.A., Hwang, J., Bottsford-Miller, J., Lu, C., Lin, Y.G., Coffey, D., Spannuth, W., Nugent, E., Han, L.Y., Landen, C.N., Nick, A.M., Stone, R.L., Coffman, K.T., Bruckheimer, E., Broaddus, R., Gershenson, D.M., Coleman, R.L., and Sood, A.K. 2010. Clinical and biological impact of EphA2 overexpression and angiogenesis in endometrial cancer. *Cancer Biology & Therapy* 10: 1306-1314.
23. Rosanò, L., Spinella, F. Di Castro, V., Nicotra, M.R., Dedhar, S., Garcia de Herreros, A., Natali, P.G., and Bagnato, A. 2005. Endothelin-1 promotes epithelial-to-mesenchymal transition in human ovarian cancer cells. *Cancer Res* 65: 11649-11657.
24. Lavery, D.N., Villaronga, M.A., Walker, M.M., Patel, A., Belandia, B., and Bevan, C.L. 2011. Repression of androgen receptor activity by HEYL, a third member of the hairy/enhancer-of-split-related family of Notch effectors. *Journal of Biological Chemistry* 286: 17796-17808.
25. Parker, B.S., Argani, P., Cook, B.P., Liangfeng, H., Chartrand, S.D., Zhang, M., Saha, S., Bardelli, A., Jiang, Y., St. Martin, T.B., Nacht, M., Teicher, B.A., Klinger, K.W., Sukumar, S., and Madden, S.L. 2004. Alterations in vascular gene expression in invasive breast carcinoma. *Cancer Research* 64: 7857-7866.
26. Zhang, P., Yang, Y., Zweidler-McKay, P.A., and Hughes, D.P.M. 2008. Critical role of Notch signaling in osteosarcoma invasion and metastasis. *Clinical Cancer Research* 14: 2962-2969.

27. Hopfer, O., Zwahlen, D., Fey, M.F., and Aebi, S. 2005. The Notch pathway in ovarian carcinomas and adenomas. *British Journal of Cancer* 93: 709-718.
28. Gotte, M., Greve, B., Kelsch, R., Muller-Uthoff, H., Weiss, K., Masouleh, B.K., Sibrowski, W., Kiesel, L., and Buchweitz, O. 2011. The adult stem cell marker Musashi-1 modulates endometrial carcinoma cell cycle progression and apoptosis *via* Notch-1 and p21^{WAF1/CIP1}. *Int. J. Cancer* 129: 2042-2049.
29. Al-Hussaini, H., Subramanyam, D., Reedijk, M., and Sridhar, S.S. 2011. Notch signaling pathway as a therapeutic target in breast cancer. *Mol Cancer Ther* 10: 9-15.
30. Roy, M., Pear, W.S., and Aster, J.C. 2007. The multifaceted role of Notch in cancer. *Current Opinion in Genetics & Development* 17: 52-59.
31. Lobry, C., Oh, P., and Aifantis, I. 2011. Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. *Journal of Experimental Medicine* 208: 1931-1935.
32. Cobellis, L., Caprio, F., Trabucco, E., Mastrogiacomo, A., Coppola, G., Manente, L., Colacurci, N., De Falco, M., and De Luca, A. 2008. The pattern of expression of Notch protein members in normal and pathological endometrium. *J.Anat.* 213: 464-472.
33. Deng, L., Broaddus, R.R., McCampbell, A., Shipley, G.L., Loose, D.S., Stancel, G.M., Pickar, J.H., and Davies, P.J.A. 2005. Identification of a novel estrogen-regulated gene, EIG121, induced by hormone replacement therapy and differentially expressed in type I and type II endometrial cancer. *Clinical Cancer Research* 11: 8258-8264.

34. McCampbell, A.S., Broaddus, R.R., Loose, D.S., and Davies, P.J.A. 2006. Overexpression of the insulin-like growth factor I receptor and activation of the AKT pathway in hyperplastic endometrium. *Clinical Cancer Research* 12: 6373-6378.
35. Marquez, R.T., Baggerly, K.A., Patterson, A.P., Liu, J., Broaddus, R., Frumovitz, M., Atkinson, E.N., Smith, D.I., Hartmann, L., Fishman, D., Berchuck, A., Whitaker, R., Gershenson, D.M., Mills, G.B., Bast, R.C., and Lu, K.H. 2005. Patterns of gene expression in different histotypes of epithelial ovarian cancer correlate with those in normal fallopian tube, endometrium, and colon. *Clinical Cancer Research* 11: 6116-6126.
36. Paik, S., Shak, S., Tang, G., Kim, C., Baker, J., Cronin, M., Baehner, F.L., Walker, M.G., Watson, D., Park, T., Hiller, W., Fisher, E.R., Wickerman, D.L., Bryant, J., Wolmark, N. 2004. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *New England Journal of Medicine* 351: 2817-2826.
37. Delassus, G.S., Cho, H., Hoang, S., and Eliceiri, G.L. 2010. Many new down- and up-regulatory signaling pathways, from known cancer progression suppressors to matrix metalloproteinases, differ in cells of various cancers. *J. Cell. Physiol* 224: 549-558.
38. Inman, G.J. 2010. Switching TGF β from a tumor suppressor to a tumor promoter. *Current Opinion in Genetics & Development* 21: 93-99.
39. Massagué, J. 2008. TGF β in cancer. *Cell* 134: 215-230.
40. Nickoloff, B.J., Osborne, B.A., and Miele, L. 2003. Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. *Oncogene* 22: 6598-6608.

41. Agrawal, N., Frederick, M.J., Pickering, C.R., Bettegowda, C., Chang, K., Li, R.J., Fakhry, C., Xie, T.X., Zhang, J., and Wang, J. 2011. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* 333: 1154-1157.
42. Stransky, N., Egloff, A.M., Tward, A.D., Kostic, A.D., Cibulskis, K., Sivachenko, A., Kryukov, G.V., Lawrence, M.S., Sougnez, C., and McKenna, A. 2011. The mutational landscape of head and neck squamous cell carcinoma. *Science* 333: 1157-1160.

Chapter Eight

Vita

Rajshi Gandhi was born in Ahmedabad, India, February 9, 1988 to Ramesh and Hansa Gandhi. She, her parents, and her brother, Chirag, moved to Corsicana, Texas in 1994. She graduated from Corsicana High School in Corsicana in 2006. In May 2010, she received a Bachelor of Science degree in Biology, with a minor in Spanish, from Southwestern University in Georgetown, Texas. Subsequently, she started working at U.T. MD Anderson Cancer Center in Houston, Texas as a Research Assistant I for the Gynecology Oncology Tumor Bank. In August 2011, she entered the Masters of Science degree program at the University of Texas Health Science Center at Houston Graduate School of Biomedical Sciences where she conducted her studies under the supervision and guidance of Dr. Russell Broadus, Department of Pathology, the University of Texas MD Anderson Cancer Center.