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MASTER OF SCIENCE

By

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Houston, Texas

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THE ROLE OF ACIDIC FIBROBLAST GROWTH FACTOR AND FIBROBLAST GROWTH FACTOR RECEPTOR 4 IN HIGH GRADE SEROUS CARCINOMA

Publication No._____

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Abstract

Background: High grade serous carcinoma (HGSC) whether ovarian, tubal or primary peritoneal, continues to be the most lethal gynecologic malignancy in the USA. Although combination chemotherapy and aggressive surgical resection has improved survival in the past decade, the majority of patients still succumb to chemoresistant disease recurrence. It has recently been reported that amplification of 5q31-5q35.3 is associated with poor prognosis in patients with high grade serous ovarian carcinoma. Although the amplicon contains over 50 genes, it is notable for the presence of several members of the fibroblast growth factor signaling axis. In particular, acidic fibroblast growth factor 1 (*FGF1*) has been demonstrated to be one of the driving genes in mediating the observed prognostic effect of the amplicon in ovarian cancer patients This study seeks to further validate the prognostic value of fibroblast growth receptor 4 (*FGFR4*), another candidate gene of the FGF/FGFR axis located in the same amplicon. The emphasis will be delineating the role of the FGF1/FGFR4 signaling axis in high grade serous ovarian carcinoma, and test the feasibility of targeting the FGF1/FGFR4 axis therapeutically.

Materials and Methods: Spearman and Pearson correlation studies on data generated from array CGH and transcriptome profiling analyses on 51 microdissected tumor

samples were used to identify genes located on chromosome 5q31-35.3 that showed significant correlation between DNA and mRNA copy numbers**.** Significant correlation between FGF1 and FGFR4 DNA copy numbers was further validated by quantitative PCR analysis on DNA isolated from 51 microdissected tumor samples. Immunolocalization and quantification of FGFR4 expression were performed on paraffin embedded tissue samples from 183 cases of high-grade serous ovarian carcinoma. The expression was then correlated with clinical data to assess impact on survival. The *in vitro* expression of FGF1 and FGFR4 was quantified by real-time PCR and western blot of six high-grade serous ovarian carcinoma cell lines and compared to human ovarian surface epithelial cells to identify overexpression. The effect of FGF1 on these cell lines after serum starvation was quantified for *in vitro* cellular proliferation, migration/invasion, chemoresistance and survival utilizing a combination of commercially available colorimetric, fluorometric and electrical impedance assays. To assess FGF1:FGFR4 specific signaling effects, FGFR4 expression was then transiently silenced via siRNA transfection and the effects cellular proliferation and migration in response to FGF1 were quantified. To identify relevant cellular pathways involved, responsive cell lines were transduced with different transcription response elements using the Cignal Lenti reporter system and treated with FGF1 with and without transient FGFR4 knockdown. This was followed by western blot confirmation for the relevant phosphoproteins. Anti-FGF1 antibodies and FGFR trap proteins were used to inhibit FGF1 mediated phenotypic changes and relevant signaling *in vitro*. To model this effect *in vivo*, orthotopic intraperitoneal tumors were established in nude mice using serous

ovarian cancer cell lines that have been previously transfected with luciferase expressing constructs. The mice were then treated with FGFR trap protein. Tumor progression was then followed via bioluminescent imaging. To exclude presence of activating mutations, the *FGFR4* gene from 43 patients was sequenced.

Results: FGFR4 DNA and mRNA copy numbers were significantly correlated and FGFR4 DNA copy number was significantly correlated with that of FGF1. Survival of patients with high FGFR4 expressing tumors was significantly shorter than those with low expression (median survival 28 vs. 55 month, $p< 0.001$). In a multivariate Cox regression model, FGFR4 expression significantly increased risk of death (H.R. 2.1, $p<0.001$). FGFR4 expression was significantly higher in all cell lines tested compared to HOSE; the OVCA432 cell line, in particular, had very high expression suggesting amplification. FGF1 was also particularly overexpressed in OVCA432. FGF1 significantly increased cell survival after serum deprivation in all cell lines. Transient knockdown of FGFR4 caused significant reduction in cell migration and proliferation *in vitro* and significantly decreased the proliferative effects of FGF1 *in vitro*. FGFR1, FGFR4 traps and anti-FGF1 antibodies did not show activity *in vitro*. OVCA432 transfected with the Cignal Lenti reporter system revealed significant activation of MAPK, NFkB and WNT pathways; western blotting confirmed these results. Reverse phase protein array (RPPA) analysis also showed activation of MAPK, AKT, WNT pathways and down-regulation of E-Cadherin. FGFR1 trap protein significantly reduced tumor growth *in vivo* in an orthotopic mouse model.

Conclusions: Overexpression and amplification of several members of the FGF/FGFR signaling axis present on the amplicon 5q31-35.3 is a negative prognostic indicator in

high grade serous ovarian carcinoma and may drive poor survival associated with that amplicon. Activation of the FGF signaling pathway leads to downstream activation of MAPK, AKT, WNT and NFkB pathways leading to a more aggressive cancer phenotype with increased tumor growth, evasion of serum-starved apoptosis, and increased migration and invasion. Inhibition of the FGF pathway *in vivo* via FGFR trap protein leads to significantly decreased tumor growth in an orthotopic mouse model.

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Introduction

High grade serous carcinoma (HGSC) is the most common malignant carcinoma arising from the ovary and fallopian tube, and second most common gynecologic cancer after corpus carcinoma in the USA [1]. It also remains the most fatal gynecologic malignancy as most patients succumb to recurrence after successful primary treatment with combined debulking surgery and chemotherapy [2].

Ovarian cancers are thought to arise from the tissues making up the female gonads. Ovarian neoplasia is broadly subdivided into epithelial tumors originating from the cuboidal epithelial cell covering the surface of the ovary, sex-cord stromal tumors originating from connective tissue elements, and germ-line tumors originating from remnant embryonic tissue. Epithelial tumors are by far the most common, accounting for more than 90% of cases.

Epithelial ovarian cancers are also subdivided into several histological subtypes as they resemble other endodermal tissues rather than the postulated tissue of origin. Mucinous tumors resemble appendiceal tumors; they predominantly produce mucin with abundant goblet-like cells dominating their architecture. Endometrioid tumors resemble endometrial carcinomas with endometrial gland-like architecture. Clear cell tumors are characterized by perinuclear clearing and production of glycogen. Transitional cell tumors are rare and resemble urothelial epithelium (table I1).

Serous tumors are the most common type of epithelial ovarian tumors. They histologically resemble the ciliated epithelium of the fallopian tube. Serous tumors arising from the fallopian tube itself and the mesothelial covering of the female peritoneum are histologically indistinguishable from serous ovarian tumors. Recently,

the origin of serous ovarian tumors has been challenged. Discovery of fallopian intraepithelial neoplasia and evidence from profiling studies now point towards fallopian tube epithelium as the tissue of origin [3].

In addition to histological subtypes, epithelial ovarian tumors are further divided into borderline tumors, low grade, and high grade carcinomas. Borderline tumors are characterized by lack of invasion, well-differentiated histology, and relatively indolent course. Low grade tumors histologically resemble borderline tumors but are invasive and are associated with a poorer prognosis. High grade tumors are the most common subtype. They are the least differentiated of the subtypes and clinically most aggressive. HGSCs are the most common of all high grade ovarian tumors accounting for 60-80% of the cases [4].

Genome profiling of epithelial tumors suggests two distinct pathways for their pathogenesis (Figure I2). Borderline tumors and low grade tumors both share a high incidence of *KRAS* and *BRAF* mutations, and lack *p53* mutations. High grade tumors, on the other hand, are characterized by *p53* mutations and lack of *KRAS* and *BRAF* aberrations. It has been suggested that borderline and low grade tumors present a continuum of neoplastic development that starts with activating mutations in the *MAPK* pathway within ovarian inclusion cysts as the precursor lesions. High grade serous tumors on the other hand are thought to derive from fallopian intraepithelial neoplasia characterized by *p53* loss of function [3].

The cornerstone of treatment of HGSC is primary surgical cytoreductive surgery with the aim of reducing tumor burden to microscopic disease [5]. This is usually followed by adjuvant combined treatment with a platinum and taxane containing chemotherapy,

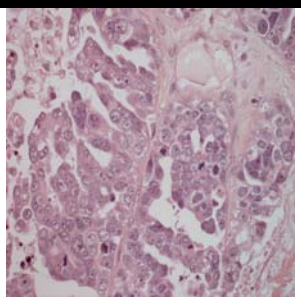
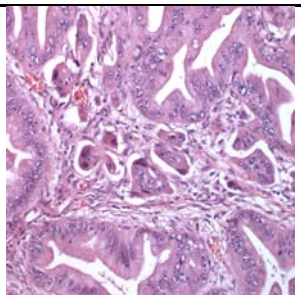
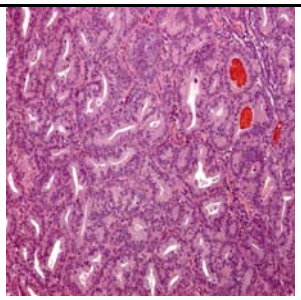
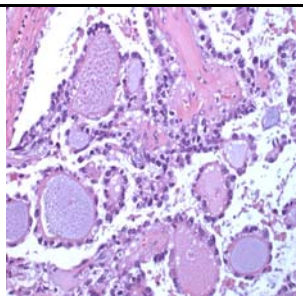
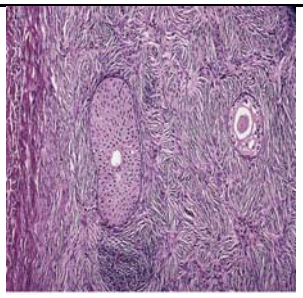
Epithelial Ovarian Tumor Type	Representative section
Serous **(resembles Fallopian tube)**	
Mucinous **(resembles GI goblet glands)**	
Endometrioid **(resembles endometrial glands)**	
Clear cell **(resembles GI endoderm)**	
Transitional **(resembles bladder urothelium)**	

Table I1 .The histologic sub types of epithelial ovarian carcinomas.

which affords 80% of patients an initial complete response and a disease free period ranging between twelve and twenty four months [6]. Most patients present in late stage advance disease (stage III-IV). Abdominal and pelvic recurrence rates are high and response to further chemotherapy is limited [7]. Attempts at introducing biologic therapeutic agents to improve outcome in this disease are ongoing [8]; however thus far, only the anti-VEGF antibody, bevacizumab, has been reported to have clinical activity in a phase III clinical trials [9].

Our group has recently reported that the amplicon 5q31-35.3 is a negative prognostic indicator in HGSC and that overexpression of fibroblast growth factor 1 (FGF1) is implicated in reducing survival [10]. It was notable that several members of the fibroblast growth factor family are also present on 5q31-35.3, including fibroblast growth factor 18 (FGF18), and the fibroblast growth factor receptor 4 (FGFR4) [11], which may also be involved in ovarian cancer progression.

Fibroblast growth factors (FGFs) are a family of small signaling proteins with a wide range of biologic effects. To date, 18 members of the FGF family have been described in mammals. All share a core 120 amino acid region and differ in structure at their carboxy & amino terminals [12]. Receptor tyrosine kinases (RTKs) are the main target by which FGF ligands induce signal transduction, although some direct nuclear signaling has been reported [13]. FGFs are expressed in a variety of tissues and have been implicated in angiogenesis [14], embryonic development/differentiation [15] and tumorigenesis [16]. FGFs have been shown to signal through autocrine, endocrine and paracrine pathways [17]. FGFs involvement in cancer biology have been well documented [18], with different members playing diverse roles related to the cancer

phenotype including tumor proliferation [19], survival [20], migration/invasion [21], EMT [22] and angiogenesis [23].

Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	65%
III	Spread IP or nodes	58%	45%
IV	Distant metastases	17%	<5%

Figure I1. Distribution of stage and prognosis in ovarian epithelial carcinoma.

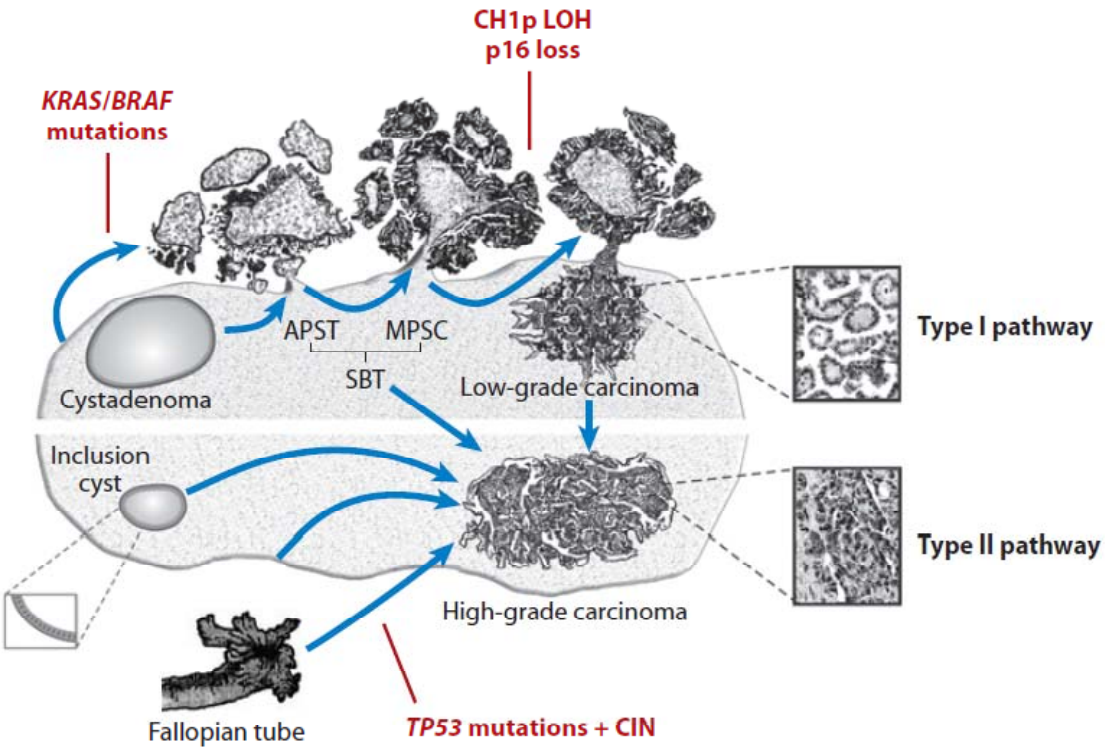


Figure I2. Illustration of the different pathways potentially leading to low versus high grade ovarian carcinoma. Abbreviations: APST, atypical proliferative serous tumor; CIN, chromosomal instability; LOH, loss of heterozygosity; MPSC, micropapillary serous carcinoma. Figure modified from reference [24] with permission.

FGF signaling is mediated through 4 transmembrane RTKs that are a product of 4 highly conserved genes, *FGFR1* through *FGFR4* [12] . Through alternative splicing, 14 variants of FGFRs have been described across the 4 FGFR classes, with only FGFR4 lacking multiple isoforms [25]. The prototypical structure of FGFR consists of 3 extracellular immunoglobulin-like domains (IgI-IgIII) which impart ligand specificity at the amino terminus, followed by a transmembrane domain and a highly conserved split tyrosine kinase domain at the carboxy end [26].

As with many RTKs, activation of the FGF signaling axis requires FGF ligand binding to and subsequent dimerization and autophosphorylation of FGFR [27]. This interaction requires presence of heparin sulfate glycosaminoglycans which acts to increase binding affinity, stabilize the ligand/ receptor dimer, and increase half life [28]. Once binding of the FGF-heparin complex occurs, receptors dimerize with subsequent autophosphorylation of the intracellular domain tyrosine residues, paving the way for activation of several intracellular signaling pathways [29].

FGFR downstream signaling leads to activation of several second messenger pathways with a variety of outcomes depending on ligand, receptor, cell and tissue type, and cross talk with other pathways [27]. The docking protein, FGFR substrate 2 (FRS2), plays a critical role in activation of MAPK/ERK and PI3K/AKT pathways downstream of FGFR. FRS2 is bound to the juxtamembrane portion of FGFR; once FGFR is activated and autophosphorylated, FRS2 is recruited and also undergoes phosphorylation by the activated receptor. The phosphorylated FRS2 recruits the adaptor proteins Sons of Sevenless (SOS) and growth factor receptor-bound 2 (GRB2), which subsequently activates RAS, downstream RAF, and eventually

MAPK/ERK pathway [26, 30]. Through GRB2 associated protein 1, the FGFR-FRS2-GRB2 complex can also bind and activate PI3K with downstream activation of Akt [31].

The phosphorylated carboxy-terminus of FGFR can also bind the SH2 domain of phospholipase C with subsequent activation of PKC pathway [32]. STAT pathway activation has been described with FGFR3 but the mechanism remains unclear [33].

Termination of FGFR signaling involves feedback inhibition by several pathways. Non-specific attenuation of FGFR signaling is mediated through RTK inhibitor pathways. These include members of the Sprouty (Spry) family, which work by inhibition of ERK activation through sequestration of GRB2 [34]. MKP3 (MAPK phosphatase 3) is another inhibitor of RTK signaling that also acts by dephosphorylating ERK [35]. A single pass transmembrane protein encoded by the *SEF* gene has been identified as specific inhibitor of FGFR1, it is thought to prevent tyrosine kinase activation by biding to the receptor, but the exact mechanism is unknown [36, 37].

Germ line mutation in the FGF pathway is involved in several skeletal dysplasias. Activating mutations on the *FGFR* genes are reported in craniosynostosis (of premature fusion of 1 or more cranial sutures) and thanatophoric dysplasia (extremely short limbs and folds of extra skin on the arms and legs) [38]. No predisposition or familial cancer syndromes have been reported with the FGF pathway. However, driver mutilations with downstream activation of the pathway are known to be driver mutation in certain types of multiple myeloma where fusion proteins produce continuous activation of the FGFR kinase domain [39]. Aberrations in the expression and mutations of FGFs and FGFRs in the solid tumors, to which HGSC belongs, have been reported to play an important role in their pathogenesis (Table I1).

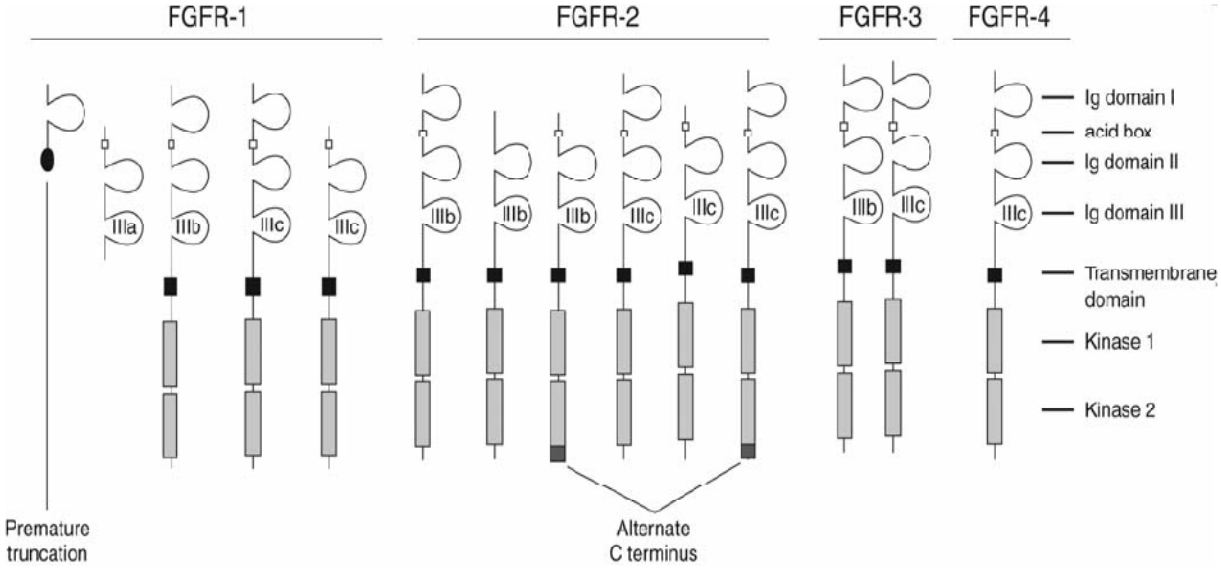


Figure I3.Structure of the 4 FGFR receptors and their different splice variants, adapted with permission from reference [25]

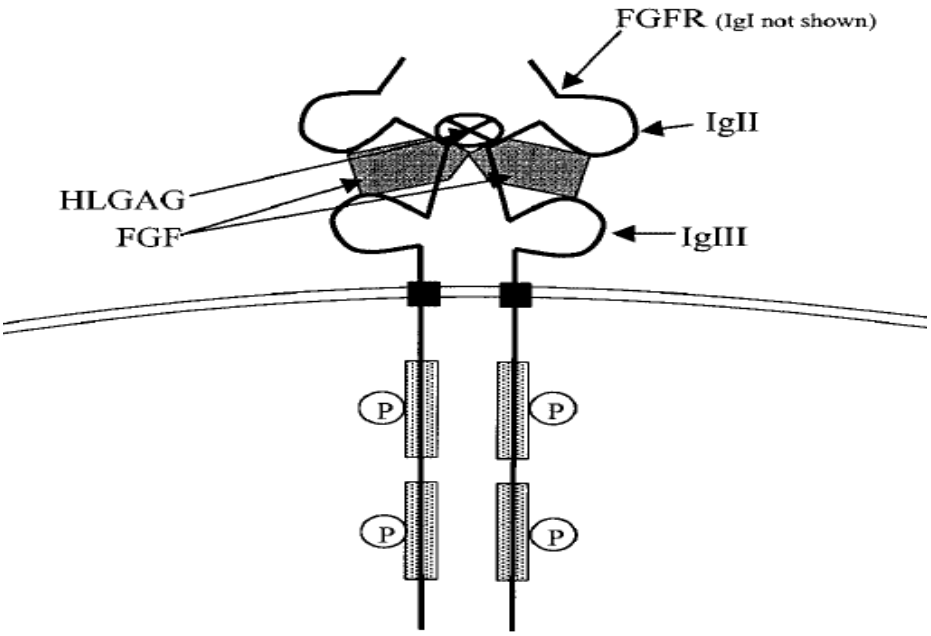


Figure I4. FGF and Heparin glycosaminoglycan complex(HLGAG) bound to FGFR causing receptor dimerization and autophosphorylation of the intracellular domain, adapted with permission from reference [25].

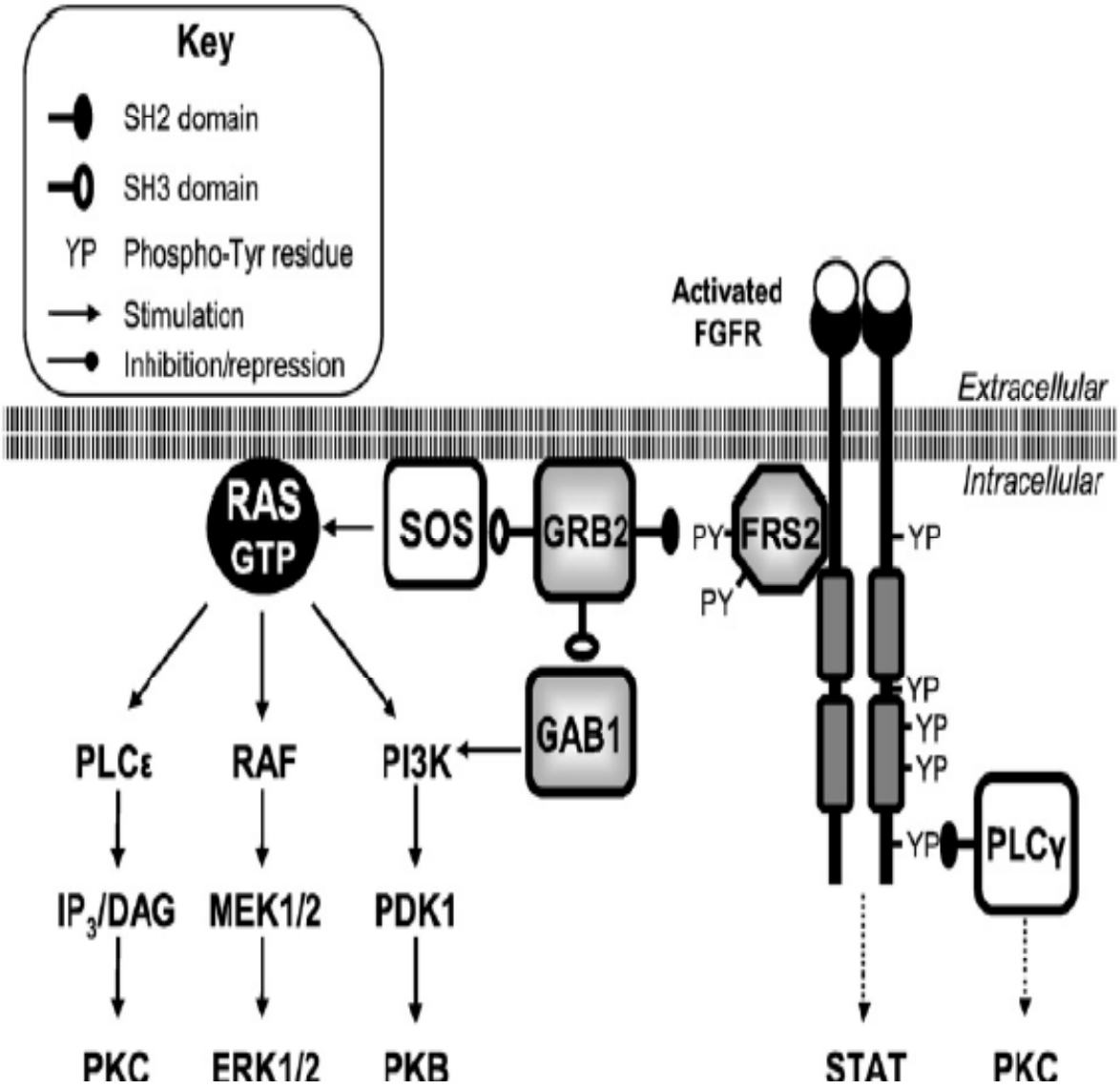


Figure I5. Downstream second messenger pathways activated by FGF signaling and associated molecules, adapted from reference with permission [40].

Activating mutations in the kinase and intermembrane domains are reported in approximately 50% of non-invasive bladder carcinomas [41]. Mutations of *FGFR2*, which are identical to the activating germline mutations found in craniosynostosis syndromes, have been described in 12% of endometrial carcinomas [42]. In breast carcinomas, the 8p11-p12 amplicon, which contains *FGFR1*, is observed in about 10–15% breast cancer patients [43]. Amplification and overexpression of *FGFR2* is

observed in about 4–12% breast tumors whilst *FGFR4* protein is overexpressed in around 30% patients [44, 45].

Gene	Cancer type	References
FGFR1		
Amplification	Breast cancer , ovarian cancer , bladder cancer and rhabdomyosarcoma	[46-50]
Mutation	Melanoma	[51]
FGFR2		
Amplification	Gastric cancer and breast cancer	[52, 53]
Mutation	Endometrial cancer and gastric cancer	[54, 55]
Germline SNP	Second intron SNP: increased incidence of breast cancer	[56, 57]
FGFR3		
Amplification	Bladder , salivary adenoid cystic cancers	[58, 59]
Mutation	Bladder cancer , cervical cancer , prostate and spermatocytic seminoma	[41, 60-64]
FGFR4		
Mutation	Rhabdomyosarcoma	[65]
Germline SNP	Coding SNP: poor prognosis breast, colon and lung adenocarcinoma	[66, 67]
FGF1		
Amplification	Ovarian	[10]

Table I2. FGF/FGFR axis aberrations reported for various solid tumors.

Activating mutations in the kinase and intermembrane domains are reported in approximately 50% of non-invasive bladder carcinomas [41]. Mutations of *FGFR2*, which are identical to the activating germline mutations found in craniosynostosis syndromes, have been described in 12% of endometrial carcinomas [42]. In breast carcinomas, the 8p11-p12 amplicon, which contains *FGFR1*, is observed in about 10–15% breast cancer patients [43]. Amplification and overexpression of *FGFR2* is

observed in about 4–12% breast tumors whilst *FGFR4* protein is overexpressed in around 30% patients [44, 45]. *FGFR2* amplification has been found in up to 10% of primary gastric cancers[55], activating mutations have also been found in FGFR2 in primary gastric cancers [68]. The SNP Gly388Arg has been reported to be a poor prognostic indicator in breast, lung, and colon carcinomas [67].

Since FGFR inhibition can reduce proliferation and induce cell death in a variety of *in vitro* and *in vivo* tumor models, inhibitors of FGFR or FGFR-dependent downstream signaling pathways may represent useful therapeutic agents. FGFR inhibition can be achieved by several approaches and both small molecule tyrosine kinase inhibitors directed against FGFR activity, and FGFR-antagonistic antibodies and trap protein have been described[40].

In undertaking this investigation, we aim to build on evidence that the amplification of FGF pathway related genes on segment 5q31-35.3 negatively impacts survival in HGSC, and to explore possible avenues to intervene therapeutically to improve outcome in those patients.

Hypothesis and Specific Aims

We hypothesize that fibroblast growth factor pathway activation is one of the main factors in conferring poor prognosis associated with amplification of 5q31-35.3 in high-grade serous carcinoma. The amplification and overexpression of *FGF1* ligand by such tumors stimulate ovarian cancer growth and survival 1) directly through autocrine signaling via several second messenger pathways or 2) indirectly through stimulating angiogenesis in a paracrine manner as previously reported. In addition, we hypothesize that *FGFR4* is another driving gene located in the same amplicon where *FGF1* is located. Binding of FGFR4 ligands, including FGF1 activates the downstream signaling pathways, which promote ovarian cancer growth and lead to poorer patient survival. The hypothesized overexpression of FGF ligands and the FGFR4 receptor in HGSC suggest that use of biologic agents targeted to that pathway may be of therapeutic benefit.

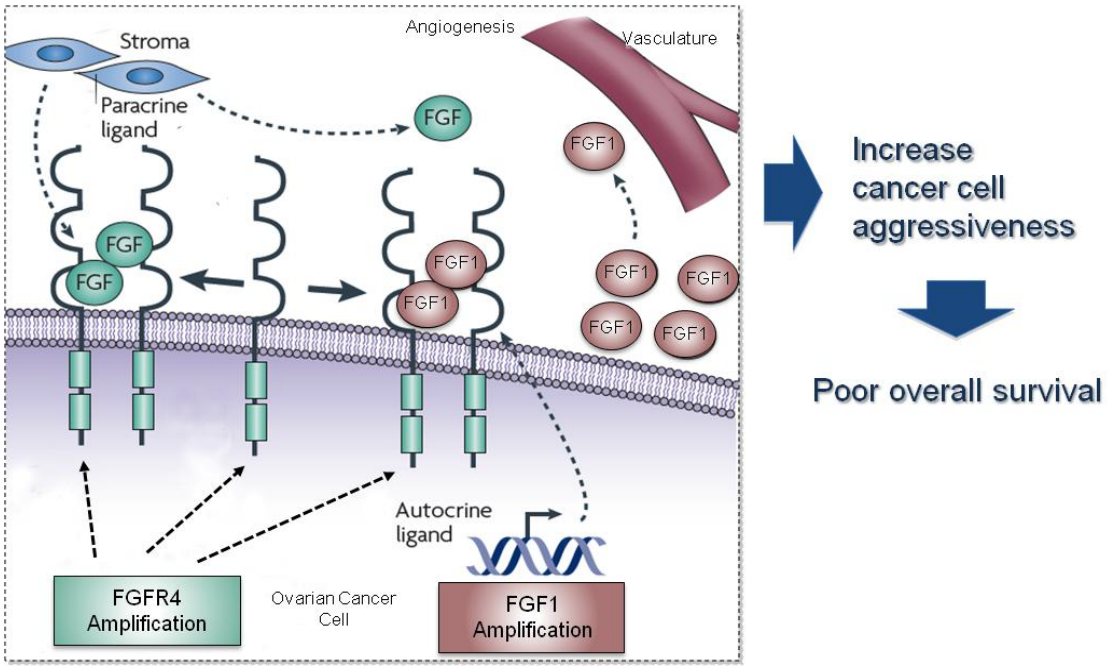


Figure H1. Illustration of central hypothesis.

The specific aims are as follows:

1. To quantify the expression of FGFR4 in HGSC, correlate its expression to that of FGF1, and determine its impact on survival.

2. To identify the mechanisms by which FGF1/FGFR4 overexpression and activation impact HGSC cell behavior.

3. To identify signaling pathways in HGSC related to the FGF1/FGFR4 axis.

4. To target the FGF1/FGFR4 axis in HGSC with suitable biologic agents to determine feasibility of therapeutic intervention.

Validation of FGFR4/FGF1 amplification in tumor samples

Based on previously reported CGH data [10], validation of gene copy number on segment 5q31-35.3 was done by quantitative polymerase chain reaction (qRT-PCR) to assess relative copy number of the genes of interest. Sixty-three DNA samples were extracted as previously described for CGH analysis [10], and amplified by GenomiPhi whole genome amplification system (Amersham, Piscataway, NJ) according to the manufacturer's instructions. qRT-PCR was then performed using primers specific for each gene using SYBR green as per manufacturer's instructions (Qiagen Sciences Inc, Germantown, MD). DNA content was normalized to that of Line-1, a repetitive element for which copy numbers per haploid genome are similar among all normal and neoplastic cells. Relative DNA copy number was determined by normalizing to that of normal human ovarian surface epithelial cells. Correlations between DNA copy number fold change and survival data were performed using Cox regression and Kaplan-Meier analysis.

Patient samples

Paraffin sections from 183 late stage high grade serous ovarian carcinoma cases were obtained from the pathology repository at the University of Texas M.D. Anderson Cancer Center under the approval of the Institution Review Board (IRB). Clinical/ pathologic data regarding age, stage, histology, overall survival, and extent of residual disease after surgery was available for these sections.

Immunolocalization of FGFR4

Tumor sections underwent deparaffinization by incubation in xylene for 5 minutes and then rehydration in decreasing concentrations of alcohol in water. Antigen retrieval was then performed by heating slides to 95^{o}C for 10 minutes in citric acid buffer (pH 6.0). The sections then underwent immunohistochemistry staining utilizing the Labvision 360 Automated Stainer (Labvision Corp, Fremont, CA). A commercially available FGFR4 antibody was used to specifically bind FGFR4 in the fixed tissue (sc-124 Santacruz biotech, Santa Cruz, CA). Parameters for the IHC cycle were as follows:

Step	**Reagent** (lab vision Corp, Fremont CA)	**Incubation time**
Endogenous peroxidase block	Hydrogen peroxide block solution	15 minutes
Protein background block	Ultra V block solution	5 minutes
Primary antibody incubation	SC-124 , 1:50 dilution	90 minutes
Secondary conjugated polymer	Ultravision LP HRP polymer	15 minutes
Chromogen application and development	DAB Plus substrate	5 minute
Hematoxylin counter stain	Mayer Hematoxylin solution	1 minute

Digital photomicrographs of representative areas were taken at 20x magnification. Quantitative FGFR4 stain intensity and localization measurements were obtained by using ImagePro Plus software version 5.1, Figure 1M (Media Cybernetics Inc., Bethesda MD).

The resulting FGFR4 stain intensity score was used to divide the patients into low and high FGFR4 expression groups using the median as a cutoff point. Survival analysis utilizing both the Kaplan-Meier modeling (with log-rank significance testing) and Cox proportional hazard model were performed to determine effect on overall survival and risk of death.

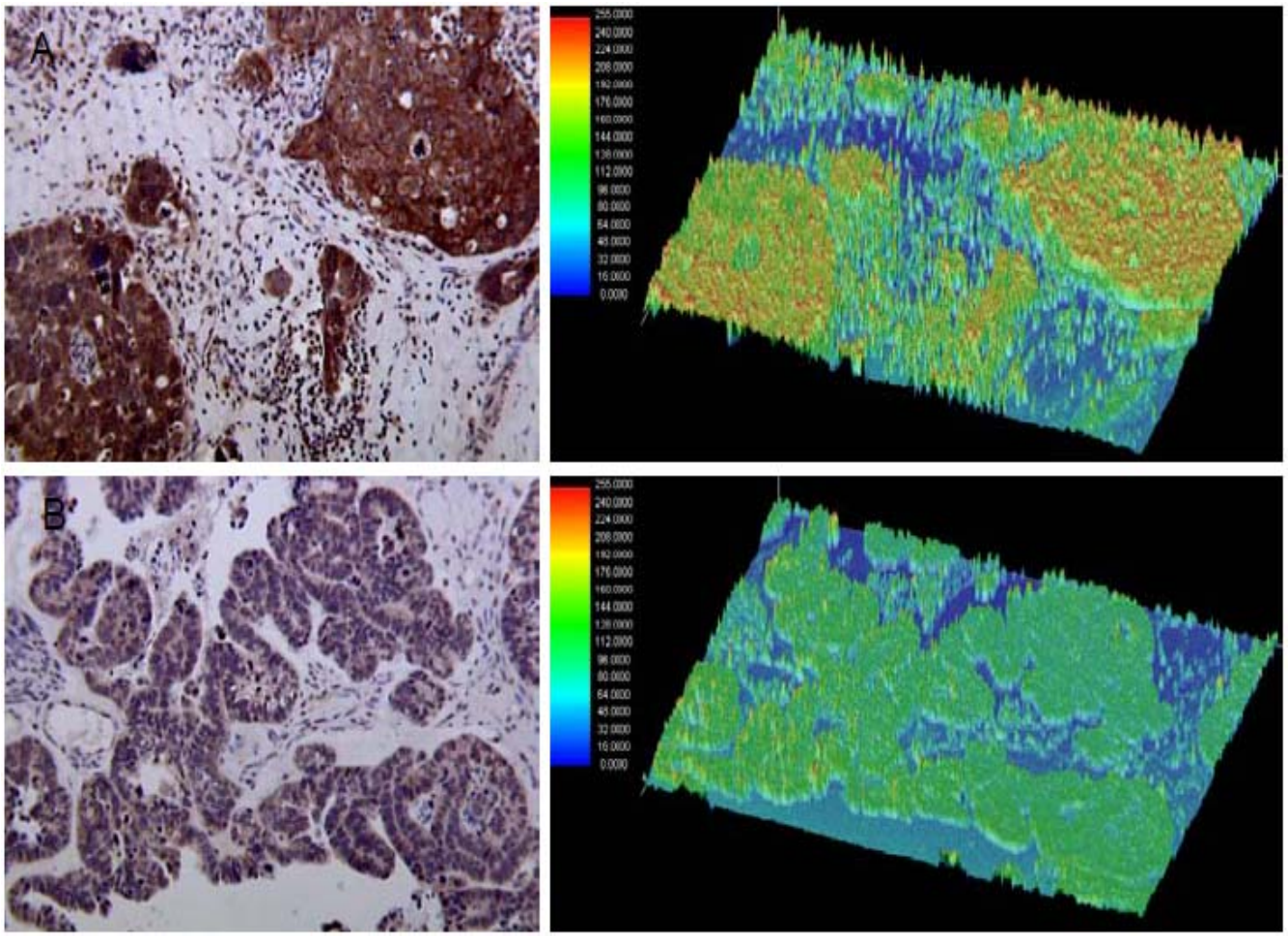


Figure M1. Computer assisted quantification of FGFR4 staining intensity in high-grade ovarian serous carcinoma. Representative images of FGFR4 IHC signal with corresponding measurement and heat map generated by software algorithm in (A) patient with overall survival of 10 months and (B) patient with overall survival of 90 months.

Cell lines and culture conditions

The derivation and source of the human epithelial ovarian cancer cell lines A2780, SKOV3ip,OVCAR-3,OV5,OVCA432 ,OVCA433 have been described previously [69-73]. All cell lines were maintained and propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 0.1% gentamicin sulfate (Mediatech Inc., Manassas, VA).

Generation of OVCA432 luciferase reporter cell lines was performed by transducing the parental cell line with lentiviral particles containing expression plasmids encoding for transcription response elements for MAPK, PI3K, WNT, NFKB, NOTCH, JAK/STAT pathways linked to a luciferase expression system (Cignal Lenti, SA Bioscience Corp, Frederick, MD) according to the manufacturer's instructions.

Generation of the luciferase expressing cell line 432luc was done via transduction with lentiviral particles containing a luciferase expression system under a CMV promoter (Genetarget, Inc, San Diego, CA) according to the manufacturer's instructions.

All experiments were performed using cells grown to 60-80% confluence. All cell lines were routinely genotyped and tested to confirm absence of mycoplasma. Passage number for cell lines did not exceed 20.

Quantification of FGFR4 & FGF1 expression *in vitro*

A) Protein quantification

To quantify FGF1/FGFR4 protein expression *in vitro* for wild type cells lines and those after treatment or knockdown, lysates were prepared from cells in log phase growth at 70-80% confluence. Cells were washed with PBS, harvested by scraping, and lysed with modified radioimmunoprecipitation assay (RIPA) lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM b-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin) supplemented with 1x protease/phosphatase inhibitor cocktail (Thermofisher Scientific, Waltham, MA). Cell lysate was centrifuged at 13,400 rpm for 10 min at 4ºC. Protein concentration was determined by a Pierce 660 nm Protein Assay kit (Thermofisher Scientific, Waltham, MA) according to the manufacturer's instructions. A total of 30 µg of protein from whole-cell lysate was subjected to SDS-PAGE on a 10% gel for 2 h at a constant 90 volts, then transferred to a nitrocellulose membrane utilizing semi-dry transfer method (Bio-Rad Labs, Hercules, CA). The membrane was then blocked with 3% bovine serum albumin solution for 1 hr at room temperature and probed with commercially available primary antibodies (Anti-FGFR4:MAB6852, dilution 1:500, Anti-FGF1:AF232, dilution 1:1000, both from R&D Biosystems, Minneapolis, MN, and Anti-beta-actin, A1978, dilution 1:10,000, Sigma Aldrich, St Louis, MO) at 4ºC overnight. The membranes were rinsed three times for 10 minutes each in tris-buffered saline (concentration of TBS?) with 0.1% Tween twenty. Incubation for the secondary antibody was done for one hour at room temperature. The secondary antibodies used were IR-680LT goat anti-mouse, IR-800CW donkey anti-goat and IR-800CW goat anti-rat (Li-Cor Biotechnology, Lincoln, NE). A 1:5000 dilution was used for all secondary antibodies. The membrane was rinsed 3 times for 10 minutes each to reduce background signal. The membranes

underwent infrared imaging on a Li-Cor Odyssey system (Li-Cor Biotechnology, Lincoln, NE). Results were visualized using packaged software, which also performed quantitative band analysis via normalization to beta-actin.

B) Messenger RNA quantification

To quantify mRNA expression of genes under investigation, cells were grown to a density of $1x10^5$ cells in six well plates; the media was then removed and washed with PBS at 4°C. RNA was then isolated by lysing and processing the cells via the Ambion Purelink RNA minikit (Invitrogen Corporation, Carlsbad, CA). Quality of collected RNA was then verified using a Nanodrop ND-1000 spectrophotometer (Thermofisher Scientific, Waltham, MA)-. cDNA synthesis was performed with the high capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). Quantitative RT-PCR (qRT-PCR): Quantitative RT-PCR was performed by TaqMan approach using the CFX96 Quantitative Real-Time PCR system (Bio-Rad Labs, Hercules, CA). *FGFR4* expression levels were determined by TaqMan assay (Hs00242558-m1) while a Cyclophilin TaqMan probe (4326316E) was used as an endogenous control. TaqMan assays were performed according to the manufacturer's suggested protocol utilizing a universal PCR master mix (Applied Biosystems, Carlsbad, CA). Packaged software from the CFX96 system was used to analyze the data.

C) Quantification of FGF1 in conditioned medium

To assess the level of FGF1 production by HGSC *in vitro,* cells were grown to 80% confluence at which point the medium was changed to serum free Opti-MEM (Invitrogen Corporation, Carlsbad, CA) and incubated for 48 h. The media was

subsequently collected and concentrated by filter centrifugation, after which ELISA for FGF1 was performed via quantakine FGF1 kit (R&D Biosystems, Minneapolis, MN).

FGFR4 gene silencing via siRNA Transfection

Two commercially available validated siRNAs targeted at *FGFR4* and a non-target scramble sequence siRNA as a control were used to perform transient knockdown of the receptor in the cell lines under investigation (Qiagen Sciences Inc, Germantown, MD). Information regarding these siRNAs is given in table M1. Screening for successful knockdown was performed at the protein level and mRNA level for the cell lines in question utilizing western blot and qRT-PCR protocol described earlier (figure M2 & M3). Transfection was carried out according to the recommended cell densities and transfection reagent concentrations as suggested for reverse transcription with the Lipofectamine RNAi MAX (Invitrogen Corporation, Carlsbad, CA) transfection reagent. The concentration of siRNA used was 10nM final concentration for all siRNA duplexes and experiments.

siRNA ID (target gene)	Target sequence	Sequence
AllStars Neg. Control siRNA (No target scramble sequence)	CAGGGTATCGACGATTACAAA	Sense GGGUAUCGACGAUUACAAAUU Anti sense UUUGUAAUCGUCGAUACCCUG
Hs_FGFR4_5 (*FGFR4*)	CCGCCTGACCTTCGGACCCTA	Sense GCCUGACCUUCGGACCCUATT Anti sense UAGGGUCCGAAGGUCAGGCGG
Hs_FGFR4_6 (*FGFR4*)	CAGGCTCTTCCGGCMGTCM	Sense GGCUCUUCCGGCAAGUCAATT Anti Sense UUGACUUGCCGGAAGAGCCTG

Table M1. siRNA oligonucleotides used in transfection experiments targeting transient knock down of FGFR4 in HGSC cell lines.

Assessment of cellular proliferation/migration

A). Endpoint assays

Proliferation was assessed utilizing the WST-1 colorimetric assay (Roche Applied Bioscience, Indianapolis, IN). Cell were plated at a density of 5,000 cells per 96 well and allowed to attach for 24 h in serum free Opti-MEM. If siRNA transfection

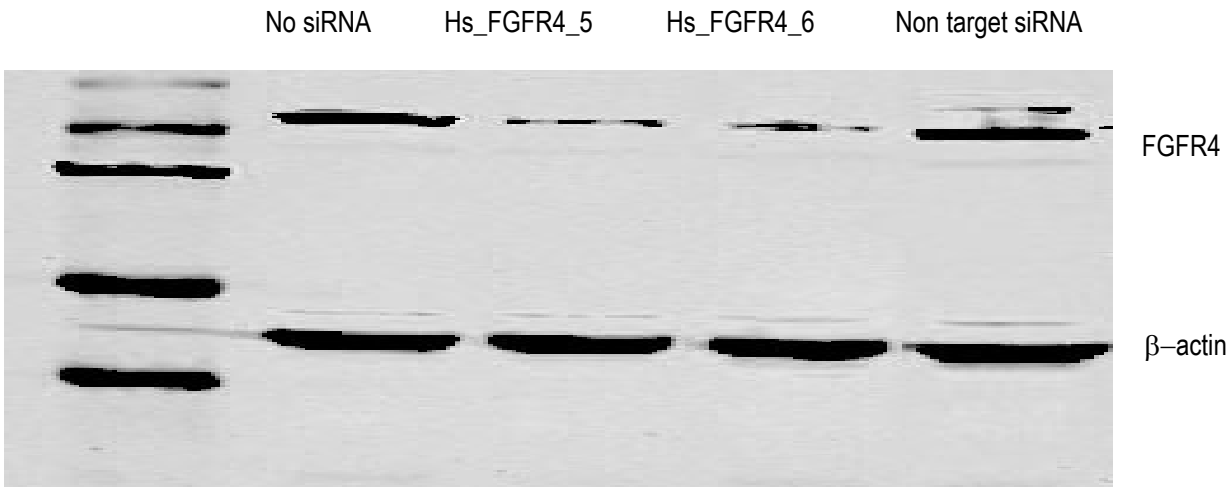


Figure M2. Knock down of FGFR4 in OVCA432 after 72 h of transfection at 10 nm final concentration with different oligonucleotides.

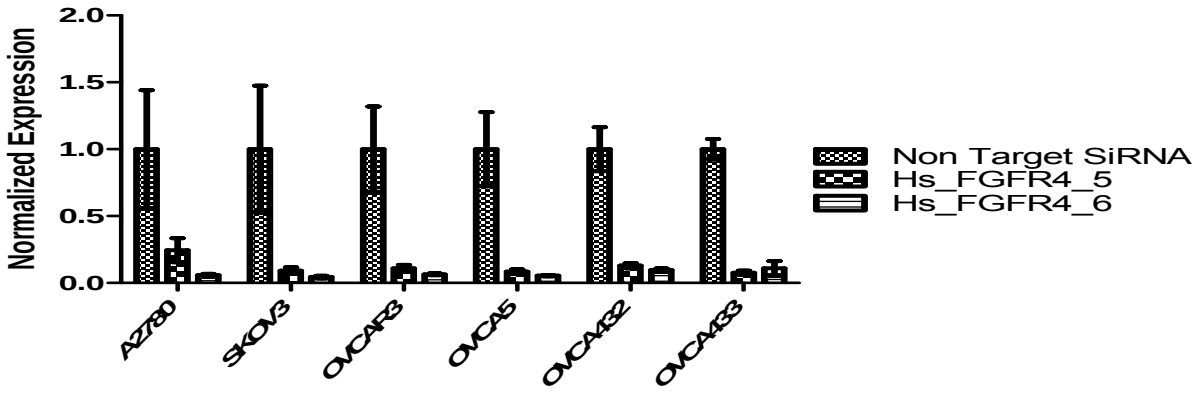


Figure M3. Knock down of FGFR4 in HGSC at the mRNA level as measured by real time quantitative PCR after 72 h of transfection at a final concentration of 10 nm.

was needed, a reverse transfection was carried at this time. After the 24 h period, media was then changed with media containing 2% FBS and any planned recombinant FGF1 treatment (R&D Biosystems, Minneapolis, MN). The cells were incubated for 3 days after which media was removed and WST-1 reagent diluted in serum free media according to the manufacturer's instruction was added and incubated for 2 h. The assay was then quantified by a spectrophotometric plate reader. All data were normalized to respective control arms.

Migration of HGSC cell lines was assessed with the ORIS migration assay kit (Platypus Technologies, Madison, WI). Cells were plated at density of 30,000 per well in serum free Opti-MEM into the 96 well plates provided with the assay, with the stoppers in place covering the central migration zone according to manufacturer's instruction. If a siRNA transfection was needed, a reverse transfection was carried at this time. Cells were left to attach for 24 h after which media containing 2% FBS and any planned recombinant FGF1 treatment was added. The stoppers were left in for a further 12 h and then removed to allow cell migration into the central zone for 12 h. Cells were then stained with Calcien AM (Invitrogen Corporation, Carlsbad, CA) according to manufacturer's instructions. Migration was assessed by measuring fluorescence in the central migration zone by a plate reader according to the protocol provided with the ORIS assay. Photomicrographs were also taken with a fluorescent microscope.

B. Real time assays

To assess cell proliferation and migration in real-time, the xCELLigence system (Roche Applied Bioscience, Indianapolis, IN) was used. The system measures electrical impedance across micro-electrodes integrated on the bottom of tissue culture plates. Impedance measurement provides quantitative information about the biological status of the cells, including cell number [74], and migration [75, 76]. Cells were plated at a density of 10,000/well cells for cell proliferation plates and 30,000/well for cell migration chambers in serum free Opti-MEM. For cell proliferation, cells were allowed to attach for 6-12 h and normalized to that point after which recombinant FGF1 was added and cell proliferation was followed on attached computer terminal and software. For migration studies, media in the upper chamber was devoid of FGF1 while media in the lower chamber contained FGF1 at various concentrations.

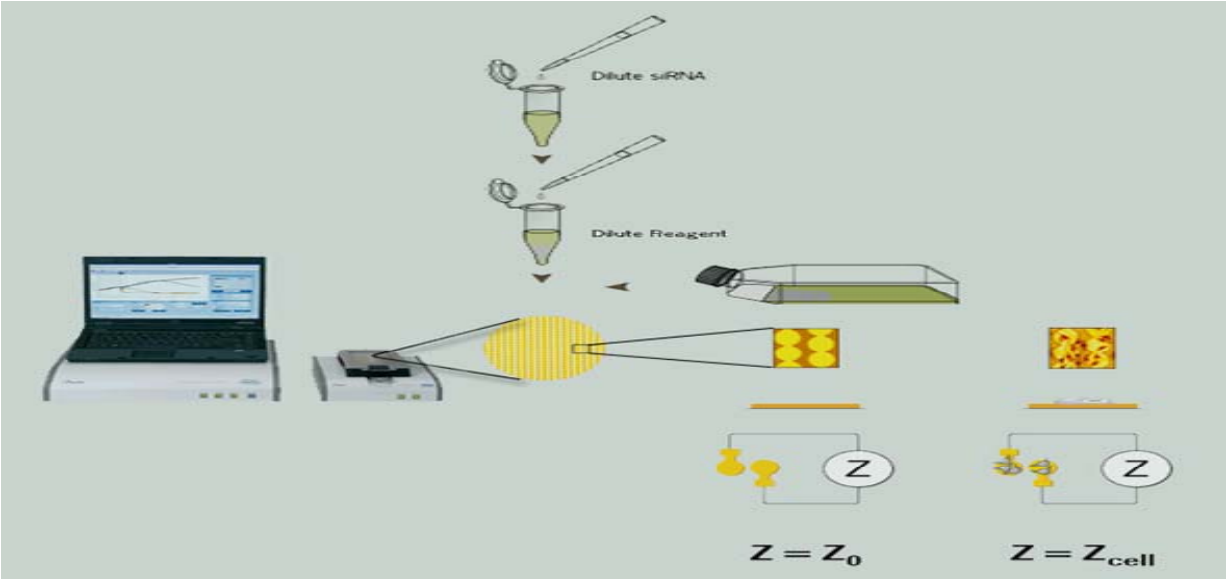


Figure M4. Real time measurement of effect of siRNA *FGFR4* knock down. siRNA and transfection reagent were mixed with cells prior to plating in wells with an electrode matrix at the bottom. The system allows realtime data acquisition of cell number as measured through changes in electric impendence across the well bottom.

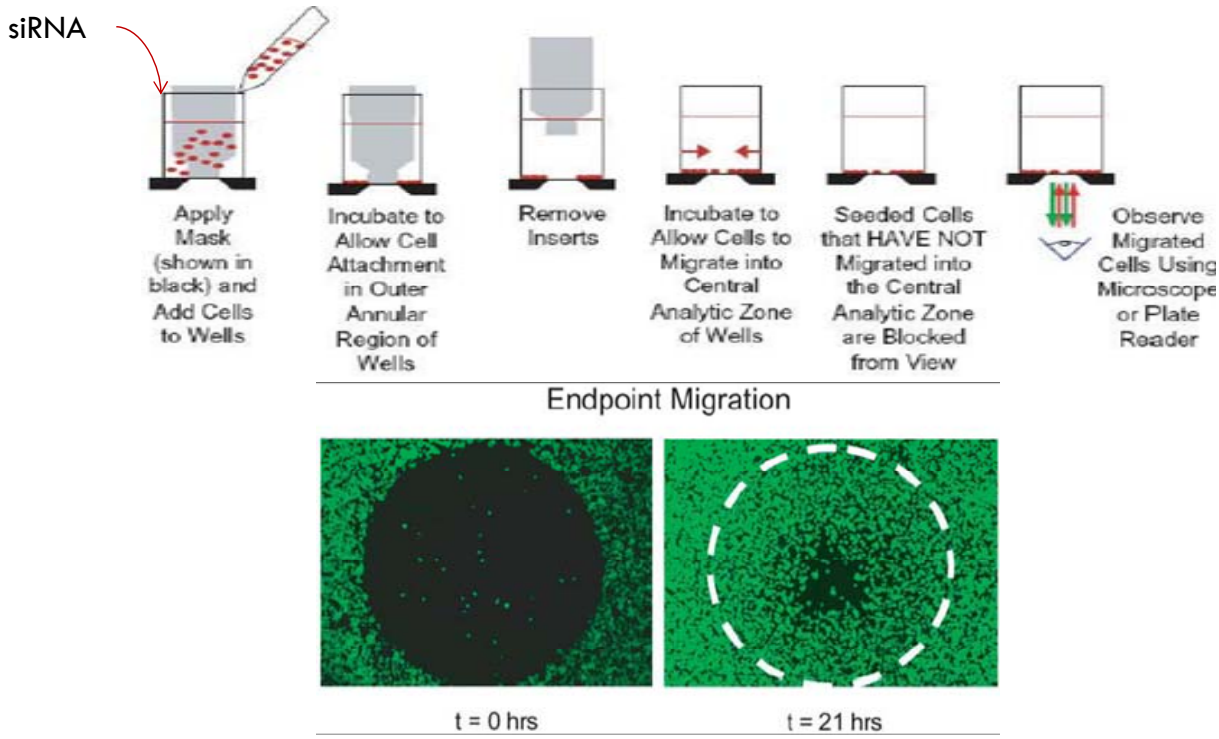


Figure M5. Use of wound healing assay to assess effects of *FGFR4* knock down on migration of HGSC. The system utilizes a stopper to create a central acellular zone into which the cells can migrate into after it has been removed. The system allows siRNA transfection to be done at the same time as cell seeding and also allows quantitative migration assessment.

Assessment of cellular signaling induced by FGF1

Cignal Lenti luciferase assay

OVCA432 cell line transected with different reporter response elements from the Cignal Lenti reporter system were plated at a density of 30000 cells/well in 96 well plates (in serum free media) and allowed to attach to for 24 h. The media was then removed. Media containing 10 ng/ml FGF1 was added and allowed to incubate for 6

hours. The media was then removed and the cells were washed with PBS once. An assay specific cell lysis buffer was then used to lyse the cell according to the manufacturer's instructions (Promega Corporation Madison, WI). The plates were then processed by a plate reader with luminescence measurement capability, and a built-in injector. The injector dispensed 50 ul of luciferin solution per well and the luminescence was measured as recommended by the manufacturers protocol (Promega corporation Madison, WI). Data were normalized to their respective control groups.

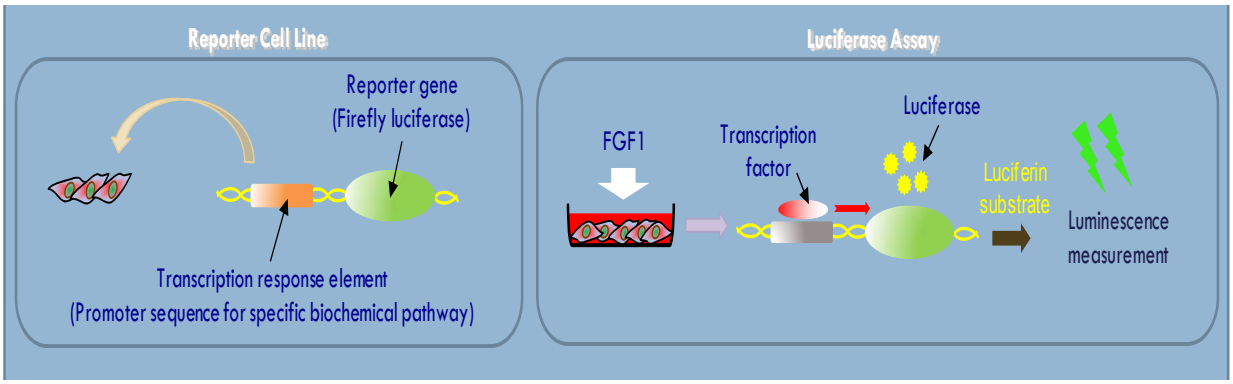


Figure M6. Use Reporter assay to screen for FGF1 pathway activation in HGSC. To screen pathway activation downstream of FGF pathway we constructed reporter cell lines in OVCA432 carrying response elements linked luciferase. Once treated with FGF 1 if the pathway is activated induced expression of luciferase will begin, in the presence of luciferin luminescence will be observed which we can measure as an indicator of pathway activation

Reverse phase protein array

OVCA432 were grown in six well plates until 80% confluent. Cells were then serum starved for 24 h. FGF1 at 10 ng/ml was then added to the experimental wells and incubated for 3 h. The cells were processed and the lysates were subjected to RPPA at the MD Anderson Core proteomics laboratory using their previously reported protocol [77, 78]

Western blotting

To confirm results of the reporter assay and RPPA, western blots of lysates from FGF1 treated cells versus serum starved controls were performed according to the protocol described earlier for FGFR4 with the substitution of different primary antibodies against the proteins of interest. These antibodies are given in the table below.

Antibody	Dilution	Manufactures
Total Erk(L34F12)	1:1000	Cell Signaling(Beverly, MA)
Phospho Erk(D13.14.4E)	1:1000	Cell Signaling(Beverly, MA)
Total Akt(40D4)	1:1000	Cell Signaling(Beverly, MA)
Phospho Akt(D9E)	1:1000	Cell Signaling(Beverly, MA)
NFKB p65 (93h1)	1:1000	Cell Signaling(Beverly, MA)
Phospho NFKB p65(C22B4)	1:1000	Cell Signaling(Beverly, MA)

Sequencing of FGFR4

DNA from 43 microdissected clinical samples was isolated and amplified as described earlier for CGH. PCR amplification of FGFR4 exons 9-16 (intermembrane and kinase domain) followed by purification and sequencing was performed as previously published [65]. The primer sequences used are given in table M2.

Therapeutic targeting of FGF pathway in vitro

An OVCA432-luciferase-transfected cell line (OVCA432-Luc) was generated in our laboratory as described earlier. An FGFR1 Fusion trap protein currently under development as a biologic therapeutic, FP-1039, was obtained from Fiveprime

Therapeutics Inc, San Francisco, CA (Figure M7). On day 1, the mice received intraperitoneal (IP) injections of $1x10^6$ OVCA432-Luc cells and were subsequently divided into 2 groups of 10 each. The experimental group received 20 mg/Kg FP1039 IP twice weekly while the control group received pooled human IgG at the same dose and schedule (Sigma Aldrich Corp, St Louis MO). Bioluminescence imaging was used

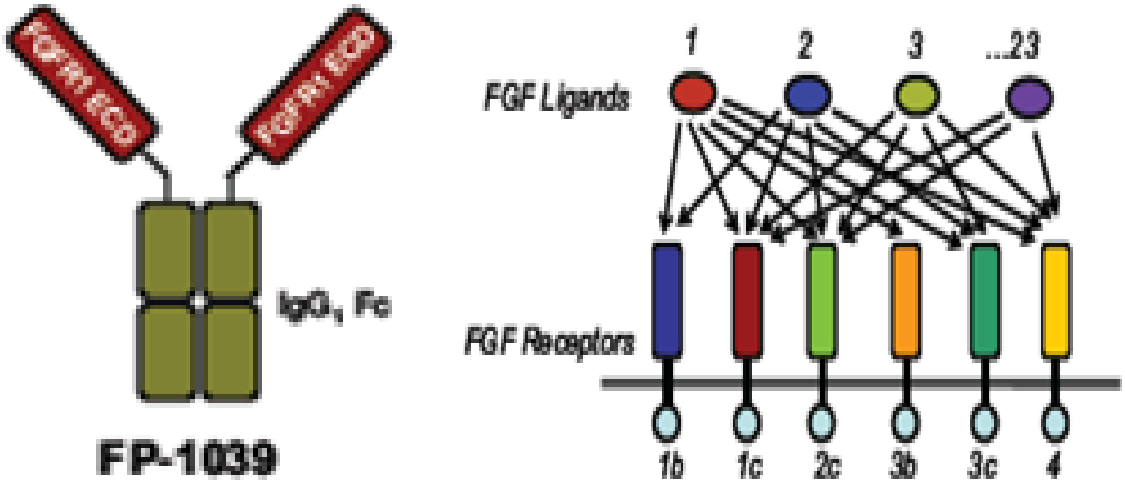


Figure M7. Structure and binding ability of FP-1039 .FP-1039 is a soluble fusion protein consisting of the extracellular domains of human FGFR1 linked to the Fc region of human Immunoglobulin G1 (IgG1) FP-1039 is designed to bind multiple FGF ligands and prevent them from activating multiple FGF receptors.

to follow tumor development in the mice on a biweekly basis. Imaging was performed on a the IVIS 100 imaging system with a data acquisition computer running Living Image software (Caliper Life Science , Hopkinton, MA). Before imaging, animals were anesthetized with a 1.5% isoflurane/air mixture and followed by injecting IP. with 15 mg/mL of D-luciferin firefly potassium salt (Caliper Life Sciences, Hopkinton, MA) in PBS at a dose of 150 mg/kg body weight. A digital gray-scale animal image was acquired followed by acquisition and overlay of a pseudocolor image representing the

spatial distribution of detected photon emerging from active luciferase within the animal. The animals were followed for a total of 8 wks after which the animals were sacrificed. Tumors were recovered, weighed, and processed for histological evaluation.

Exon 9 -10	
Forward	5'-GCTGGGAGGGACTGAGTTAG-3’
Reverse	5’-TGGAGAAAGTCCAGCCTCAG-3’
Exon 11	
Forward	5'- CTACCTCTCGACCCACTATG-3’
Reverse	5’- GTCTTGCCATGTTGCCCAGG -3’
Exon 12	
Forward	5'- GATTCAGCCCTAGACCTACG-3’
Reverse	5’- CACTCCACGATCACGTAC -3’
Exon 13	
Forward	5'- CAACCTGCTTGGTGTCTG -3’
Reverse	5’- GGAAAGCGTGAATGCCTG -3’
Exon 14	
Forward	5'- TGGTGTGTGCTCAACTCCAG-3’
Reverse	5’- GTACACCCGGTCAAACAAGG -3’
Exon 15	
Forward	5'- CCAGCAACGTGAGGGAGATG-3’
Reverse	5’- CCAAATCTGAAGGAGCCCTCG -3’
Exon 16	
Forward	5’-TGTCCTACCCCACAAAAAGG-3’
Reverse	5’-AGGAGGACGAGGAGTTGTTG -3’

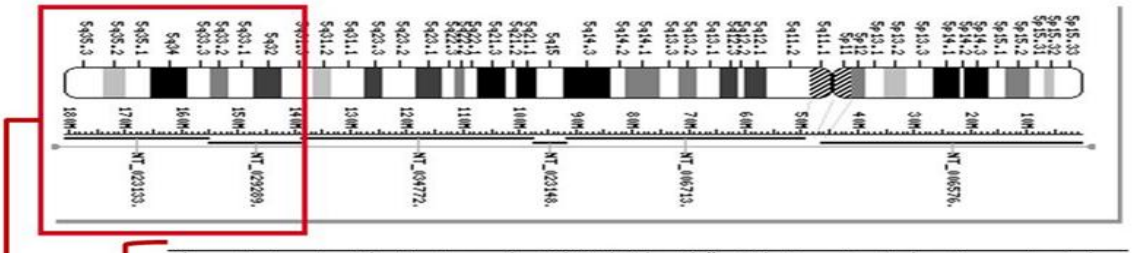
Table M2. Primer sequences for FGFR4 exons 9-16

Statistical Analysis

SPSS version 17 (IBM Corporation Somers, NY) was used to perform all statistical test. Two-tailed Student's t-test was used to test differences in sample means for data with normally distributed means. Mann-Whitney U test was used alternatively for non parametric data. Kaplan-Meier survival curves were generated and compared using a 2-sided log-rank statistic. The Cox proportional hazards model was used for multivariate analysis. Pearson's correlation coefficient was used to test linear associations. A p value < 0.05 was considered statistically significant.

FGF1 and *FGFR4* DNA copy number are significantly amplified in HGSC

Chromosome segment 5q31-35.3 was previously reported to be amplified and related to poor prognosis by our group in high grade serous ovarian carcinoma [10]. A correlation study between CGH and transcriptome profiling generated from 64 independent microdissected HGLS ovarian tumor samples identified 17 genes that showed significant concurrence between DNA and mRNA copy numbers (Figure R1) in addition to *FGF1* that we previously described. *FGFR4* located in the same amplicon was found to show significant correlation between DNA and mRNA copy number, further study using qRT-PCR analysis also showed significant positive correlation between DNA copy number of *FGF1* and *FGFR4*. (Figure R2).



Chr	Start	LocusLink	Gene Name	Clone ID (aCGH)	Probe set (Affymetrix)	Pearson *R*	*P* value	Spearman *R*	*P* value
5	140835754	5098	PCDHGC3	200017866	205717_x_at	0.300	0.075	0.395	0.018
5	141326622	9604	RNF14	200015197	243345_at	0.338	0.047	0.316	0.065
5	141953309	2246	FGF1	200006064	205117_at	0.582	<0.001	0.381	0.029
5	145698870	5459	POU4F3	200016022	208554_at	0.472	0.004	0.315	0.062
5	150460864	309	ANXA6	200012152	244250_at	0.436	0.009	0.393	0.020
5	150612838	2760	GM2A	200009479	33646_g_at	0.394	0.019	0.325	0.057
5	154178248	10826	C5orf4	200001890	48030_i_at	0.464	0.006	0.287	0.100
5	154218392	9337	CNOT8	200003673	202163_s_at	0.372	0.028	0.391	0.021
5	162862810	27430	MAT2B	200005116	229284_at	0.681	0.000	0.470	0.005
5	168943356	54908	CCDC99	200004166	221685_s_at	0.462	0.005	0.470	0.004
5	170779273	8817	FGF18	200004940	214284_s_at	0.112	0.522	0.342	0.045
5	172343396	8992	ATP6VOE	200003390	200096_s_at	0.531	0.001	0.505	0.003
5	176446528	2264	FGFR4	200007639	211237_s_at	0.321	0.050	0.297	0.083
5	177962273	57396	CLK4	200009252	228751_at	0.472	0.017	0.397	0.050
5	178473475	9509	RANGAP1	200012248	214454_at	0.456	0.008	0.225	0.206
5	180150155	4245	MGAT1	200014247	201126_s_at	0.502	0.002	0.460	0.005
5	180553532	81786	TRIM7	200019956	239694_at	0.509	0.002	0.355	0.034

Figure R1. qRT-PCR validation of genes present on segment 5q31-35.3 from 51 microdissected tumor samples. Both *FGF1* and the *FGFR4* genes are significantly amplified and over-expressed in this area of the chromosome, which is linked to poor survival in high grade serous ovarian cancer patients [10].

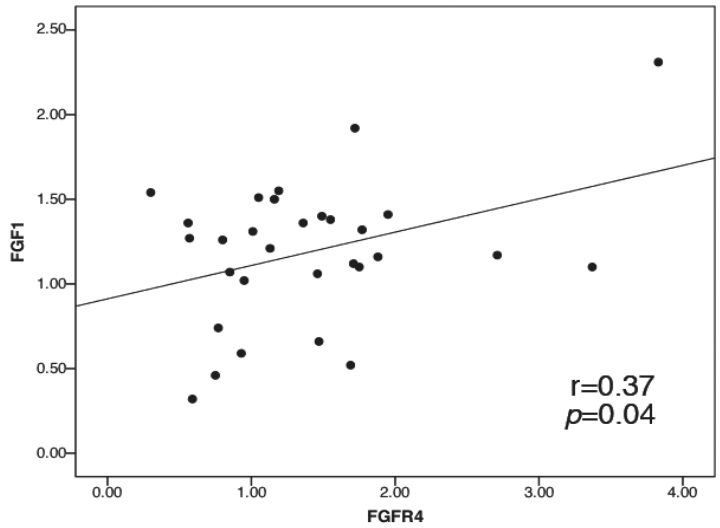


Figure R2. DNA copy number of *FGF1* and *FGFR4* genes as determined by quantitative PCR from 51 micro dissected tumor samples. Significant positive correlation is noted by person correlation (r=0.37, *p<0.05,*).

The FGFR4 protein is overexpressed in HGSC and is related to poor survival

Immunohistochemistry staining of FGFR4 in both normal ovarian surface epithelium and fallopian tube demonstrated lower expression compared to stained tumor (figure R3). FGFR4 expression score was found to correlate negatively with survival(*r*=-0.49 p<0.001, (Figure R4). The high FGFR4 Expression cohort demonstrated significantly decreased survival in Kaplan-Meier survival analysis were median survival was 24 months compared with 55 months in the low expression cohort. Cox proportional hazard model after stratifying for age and debulking status also demonstrated increased risk of death with high FGFR4 expression (HR 2.1 *p*<0.01,Figure R5).

The FGFR4 and FGF1 proteins are over expressed *in vitro* in HGSC

Western blotting demonstrated a minimum 40 fold over expression of FGFR4 and FGF1 proteins in HGSC cell line lysates compared to human surface epithelial cells

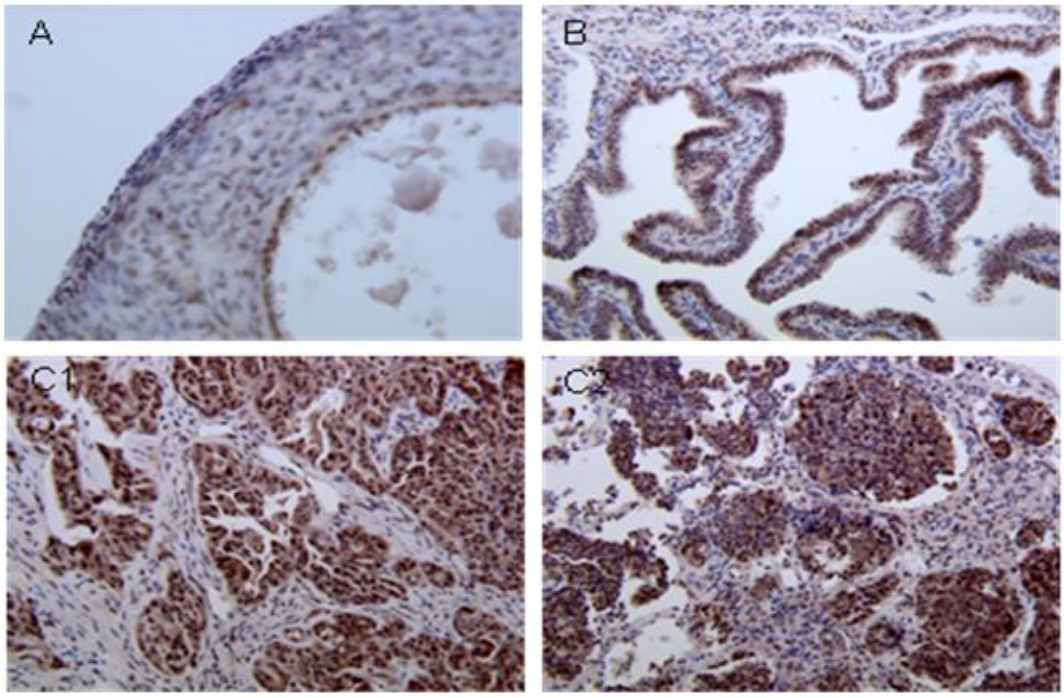


Figure R3. Overexpression of FGFR4 in high grade serous ovarian carcinoma. Expression of FGFR4 in high grade ovarian cancers (C1, C2) compared with ovarian surface epithelium (A) and fallopian tube epithelium (B) is demonstrated with overexpression being noted.

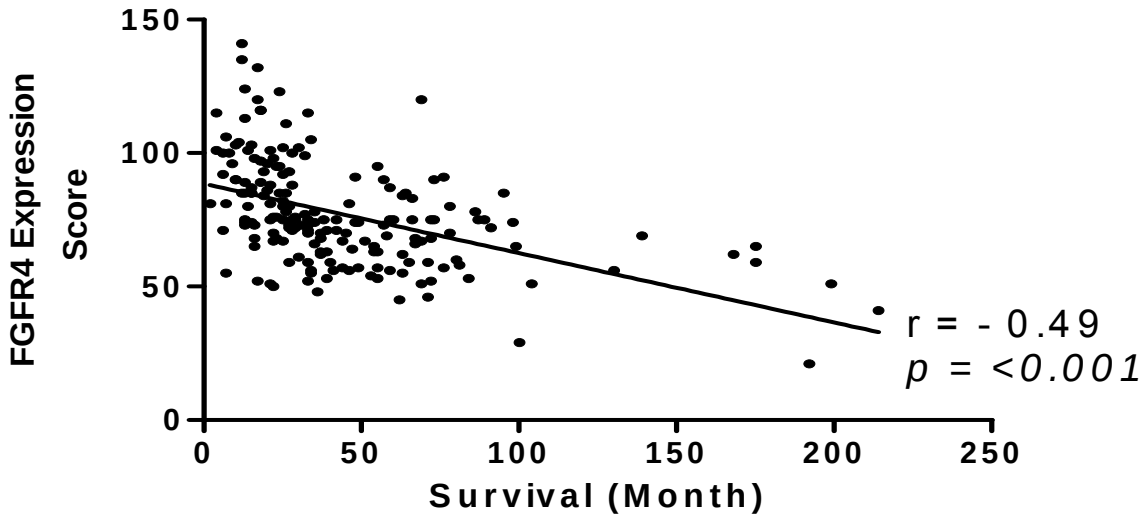
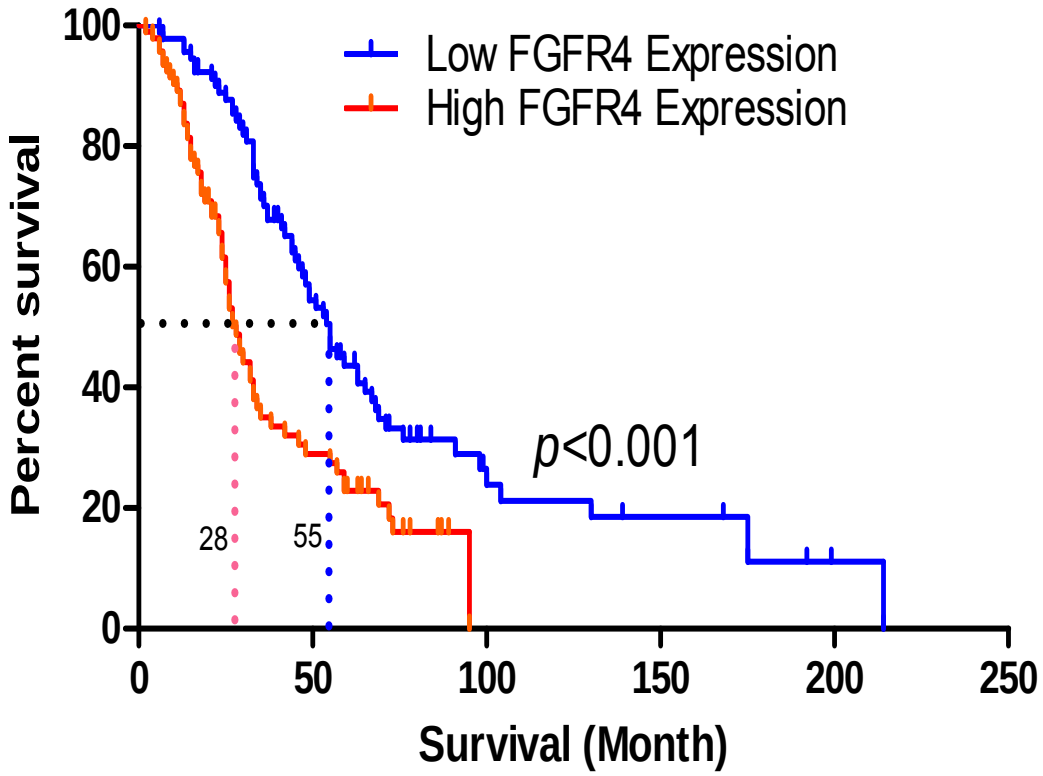


Figure R4. Correlation of FGFR4 expression score with patient survival. 183 HGSC tumor sections were scored with a computer algorithm in arbitrary units for FGFR4 staining intensity (0=lowest – 255=highest). A significant negative correlation is noted.

A

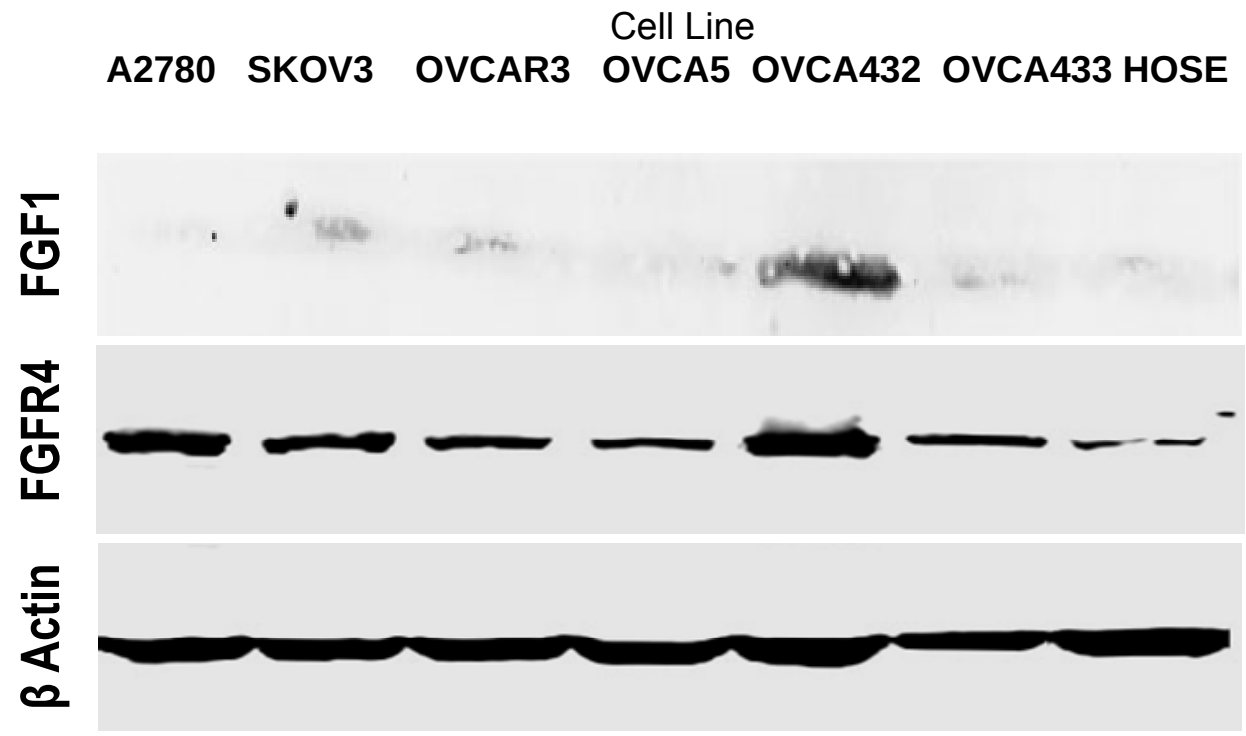


B

Factor	Hazard ratio (95%CI)	p value
optimal debulking	**0.5**(0.32-8.4)	<0.01
Age	**1.0**(0.9-1.1)	N.S
High FGFR4 expression	**2.1**(1.4-3.0)	<0.01

Figure R5. FGFR4 overexpression is associated with poor survival in advanced stage high grade serous ovarian carcinoma. (A) Kaplan-Meier survival function for 183 patients with HGSC divided into low and high FGFR4 expression based on a median expression score cut-off. High FGFR4 expressors median survival was 24 months compared with 55 months in the low expression cohort. Correlation with survival is maintained after stratification for age and debulking status. (B) Cox proportional hazard model indentified high FGFR4 expression as an independent risk factor for death in HGSC.

A



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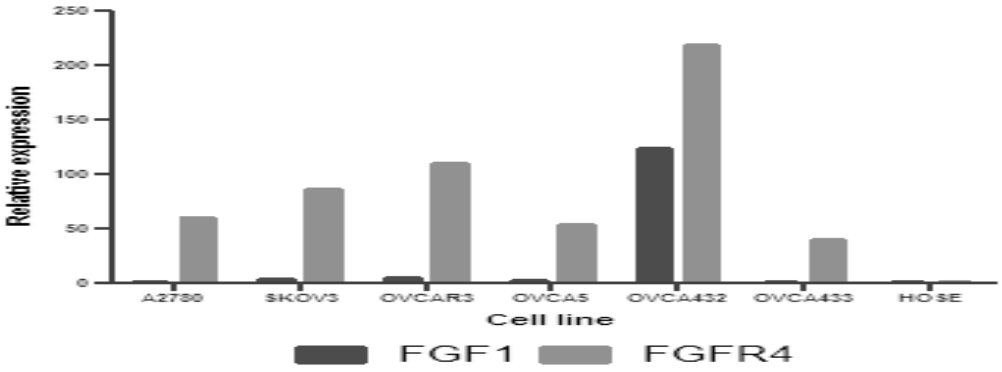


Figure R6. Expression of FGFR4/FGF1 in high grade ovarian cell lines.(A) Western blots demonstrating relative expression of FGF1/FGFR4 and β-actin in 6 HGSOC cell lines and human surface epithelial cells; **(B)** Protein expression normalized to actin from the immunoblots shown in (A).

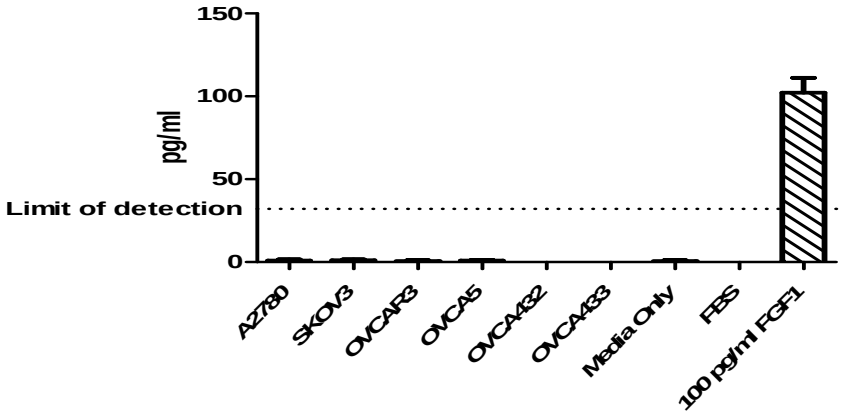


Figure R7. FGF 1 in conditioned media from HGSC cell lines in vitro (A) Cells were plated on 100 mm plates until 70% confluent, serum containing medium was removed and Opti-MEM media was added, incubation for 48 hours was then done, media collected, concentrated 50x and then subjected to FGF1 ELISA, none of the cells lines demonstrated any measurable FGF1 production in the media.

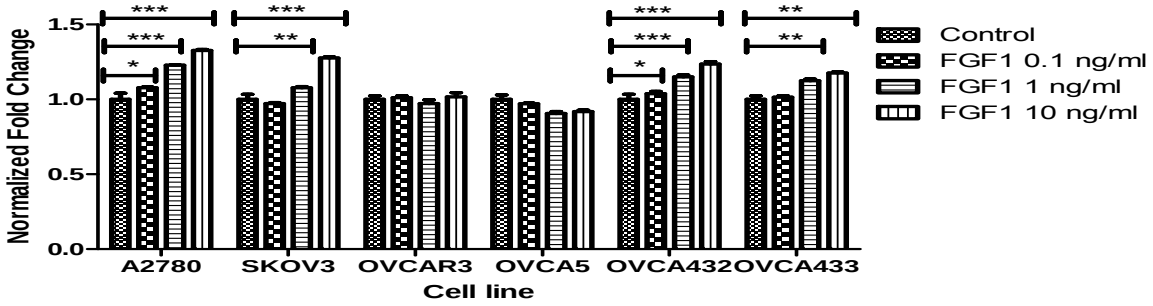
(Figure R6). Both FGFR4 and FGF1 expression were highest in the OVCA432 cell line suggesting possible concurrent amplification of 5q 31-35.3. FGF1 was not detectable in conditioned medium (Figure R7) in any of the cell lines tested.

Exogenous FGF1 treatment increased proliferation and survival in HGSC and activated multiple signaling pathways *in vitro*

Exogenous FGF1 significantly increased proliferation by 11-54% in 17 % of cell lines tested, survival was also increased by a mean of 22% in 4 out 6 cell lines *in vitro* (Figure R8 and R9-A). Treatment with exogenous FGF1 significantly increased measured luminescence attributed to activation of the MAPK, WNT and NFkB pathways by 41-53% in the Cignal Lenti OVCA432 reporter assay, no significant change was noted in the remainder of the tested pathways (Figure R8). Activation of the pathways was verified by western blotting (Figure R13). Reverse phase protein array also indentified several proteins involved in cellular signaling that showed increased phosphorylation in response to FGF1 treatment including MAPK, PI3K and GSK3α. In addition, up regulation of BCL-xL and down regulation of E-cadherin was

shown (Figure R12 & Table R1**)**. *In vitro* transfection of FGFR4 targeted siRNA was successful in abrogating the effect of exogenous FGF1 *in vitro* both on cell behavior and cell signaling (Figure.R9-B and R11) FGF1 did not increase cellular migration *in vitro* in any cell line tested. Effects of selected FGF1 inhibitors and FGFR4 knock down are shown in Figure R14. With the exception FGFR4 knockdown, none of the FGF1 or FGFR inhibitors showed activity *in vitro.* However, FGFR4 trap protein was able to block the effects of exogenous FGF1 on OVCA432.

A



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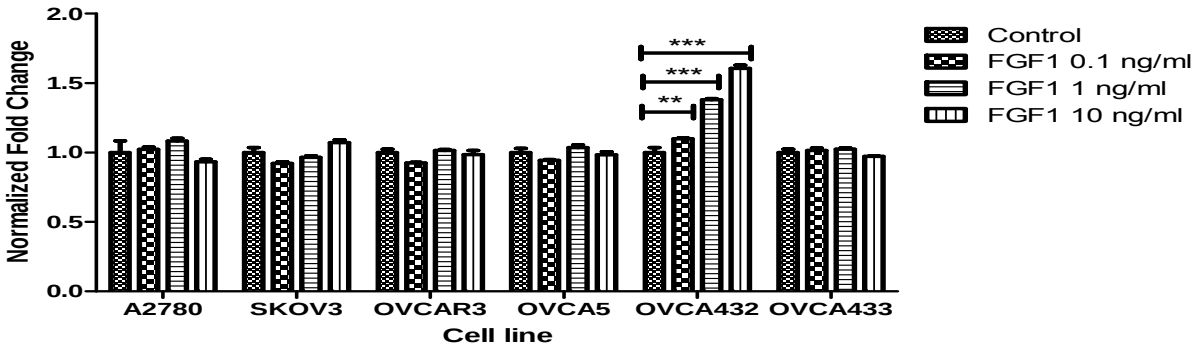
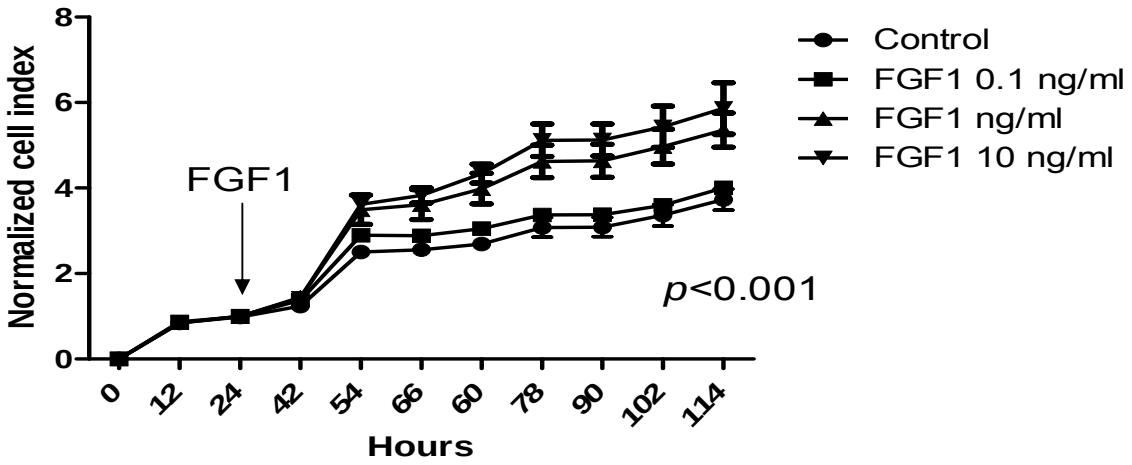


Figure R8. Effects of exogenous FGF1 on HGSC survival and proliferation in vitro. (A)Survival of HGSC cells incubated in serum free media for 72 hours with different concentration of FGF1 as measured by WST 1. Several cell lines demonstrate increased survival in a dose dependent fashion (B) Proliferation of HGSC cells, serum starved for 24 hours, and incubated for 24 h afterwards with varying concentrations of FGF1 with 2% FBS(all data points normalized to each cell lines control *=p<0.05, **=p<0.01, ***=p<0.001)

A



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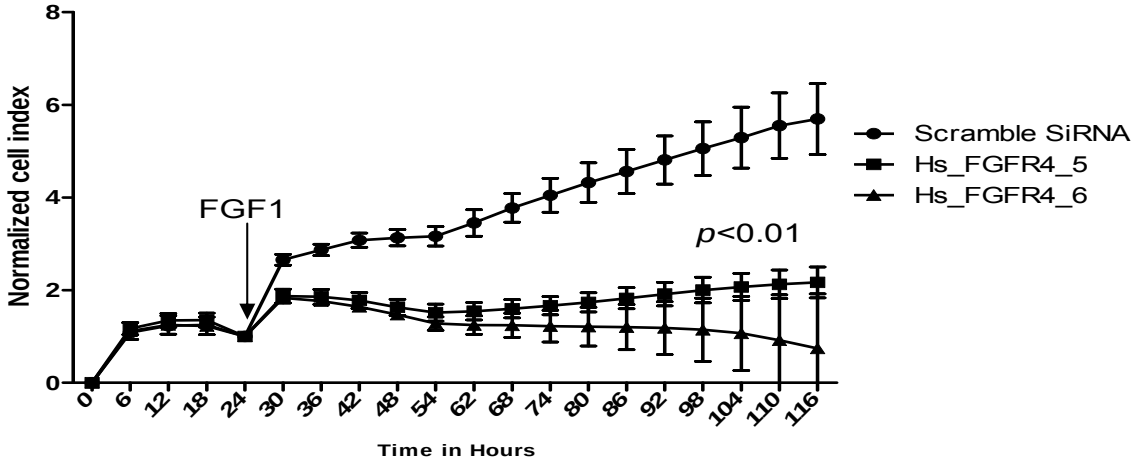


Figure R9. Proliferation of OVCA432 under different concentrations of FGF with and without transient *FGFR4* knockdown (A) OVCA432 was plated in media containing 2% FBS for 24 hours on the XCELLigence array to allow attachment, data where normalized to the 24 hr time point after which the media was supplemented with FGF1 in different concentrations and the cells were followed for 84 hours using changes in electrical impendence to generate a cell index reflecting the number of cells present. FGF1 was noted to cause increased proliferation in a dose dependant manner(B) The experiment was then repeated with prior forward transient transfection with scramble siRNA in the 24 period prior to addition of FGF1 at 10 ng/ml and 2% FBS to the medium, scramble siRNA demonstrated significantly increased proliferation compared to the two *FGFR4* Knockdown sequences

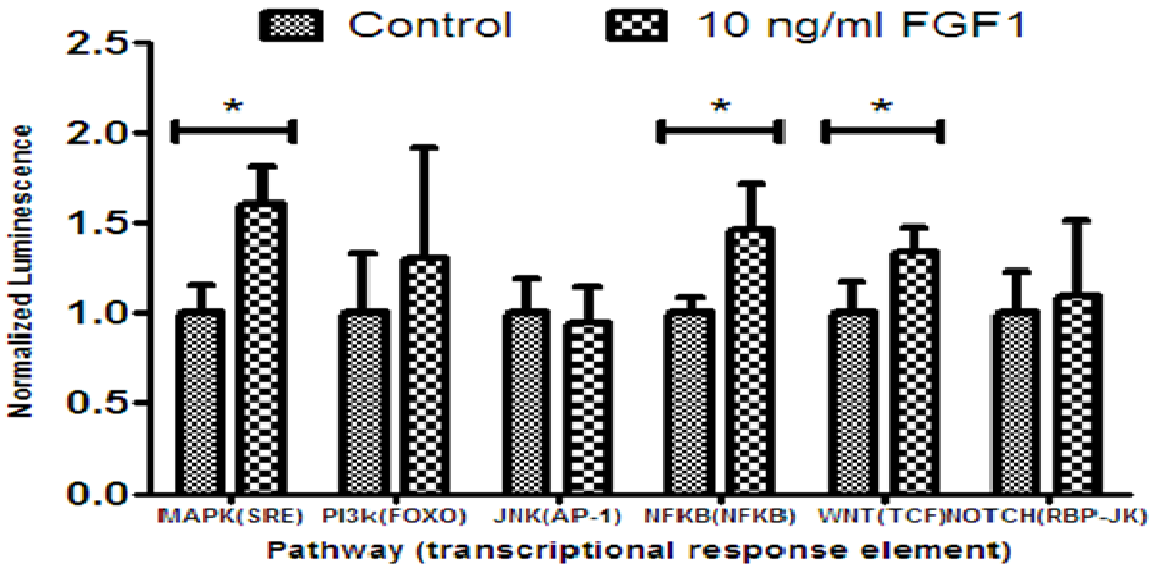


Figure R10. FGF1 effect on pathway activation in HGSC. Activation of signaling pathways in OVCA432 was screened by treating HGSC cell lines expressing transcription response elements linked to a luciferase reporter system with FGF1 and measuring subsequent light emission. MAPK , NFκB and WNT pathway demonstrated significant increases in activity (* = p<0.05 , **=p<0.01,***=p<0.001)

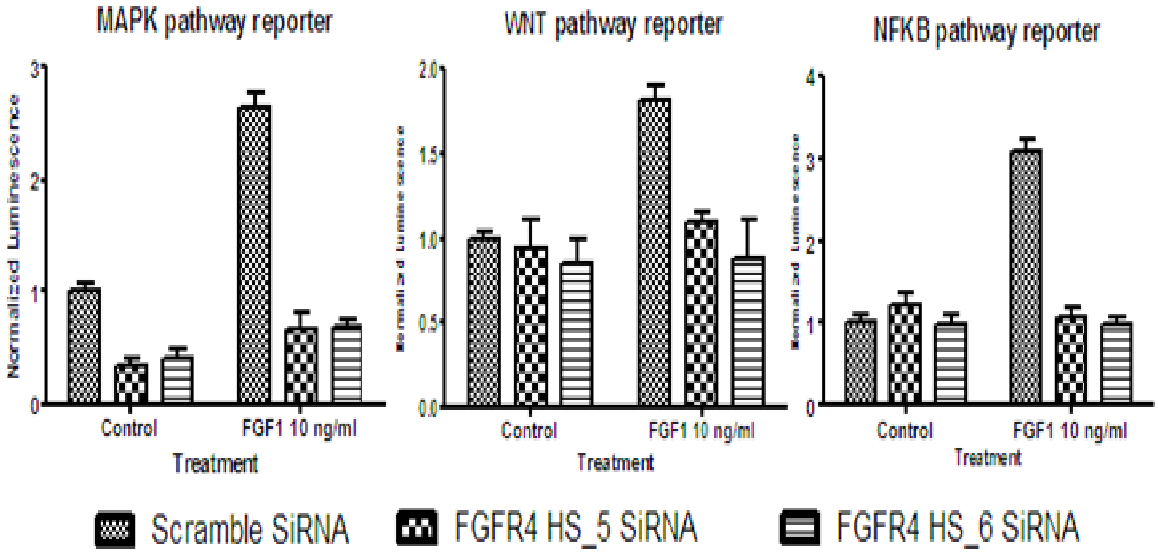


Figure R11. Transient *FGFR4* knockdown reduced signaling activation triggered by FGF1. *FGFR4* targeted siRNAs successfully inhibited increased pathway activation measured by luminescence in the luciferase reporter system previously described.

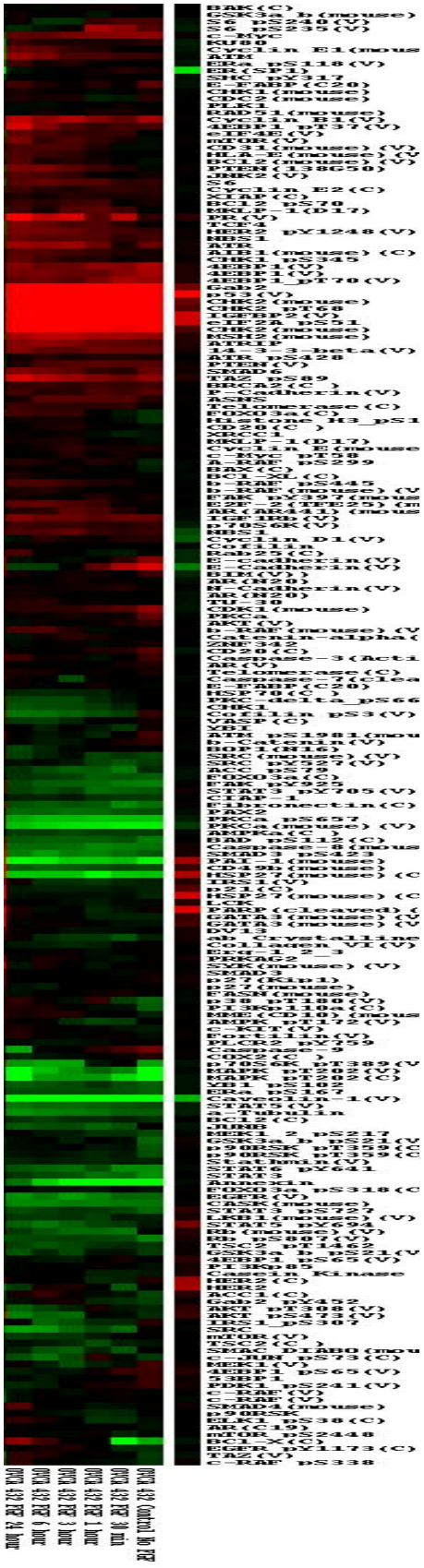


Figure R12. Reversers phase protein array of OVCA432 treated at several time points. The array used 209 antibodies to probed for changes in expression levels of protein lysates After treatment with FGF1.Activation of MAPK , PI3K and WNT pathway is noted. BCL-xL is also up-regulated. E-Cadherin and cyclin B1 are down regulated.

Up regulated Proteins	Fold change	Down regulated Proteins	Fold change
Phosphorylated MAPK(T202)	2.91	E. Cadherin	0.51
Bcl-xL	1.83	Cyclin B1	0.44
Phosphorylated GSK3α(S21)	1.54		
Phosphorylated PI3K (110a)	1.51		

Table R1. Up-regulated and down regulated proteins as shown by RPPA after treatment with FGF1 at 10 ng/ml for 3 h

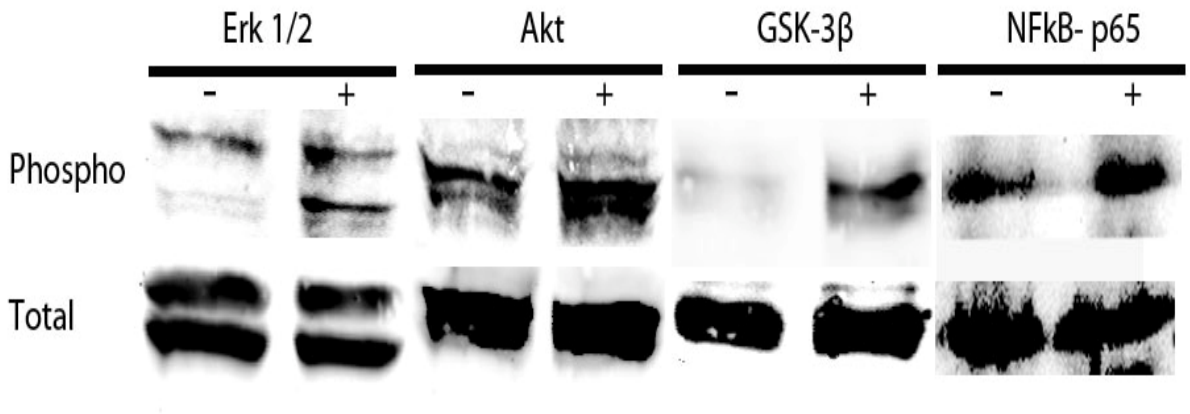
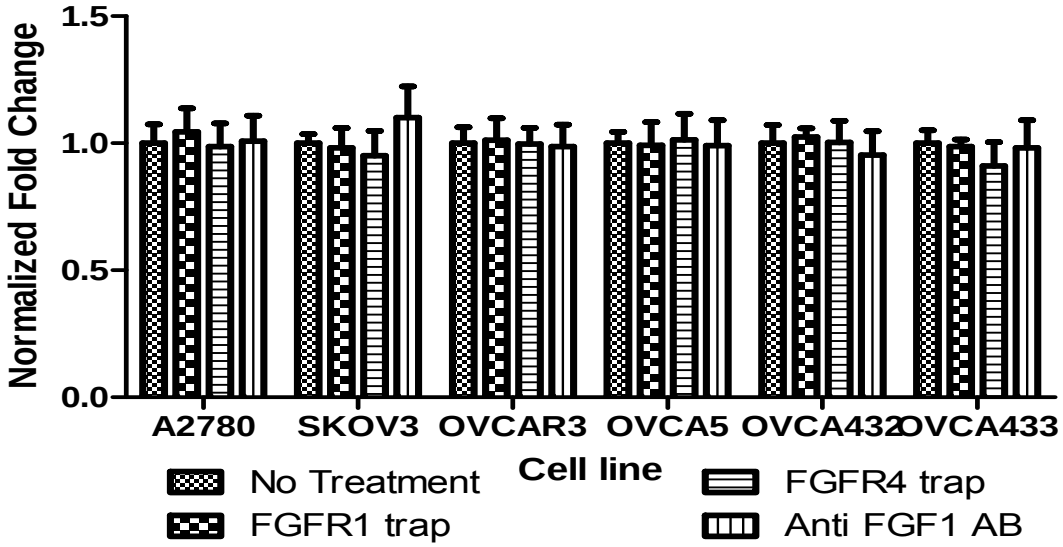


Figure R13. Pathway activation in OVCA432 treated with FGF1. Western blots demonstrating change in phosphorylated protein levels in OVCA432 cells treated with FGF1 at 10 ng/ml for 1 hr (+) versus control (-).

A



B

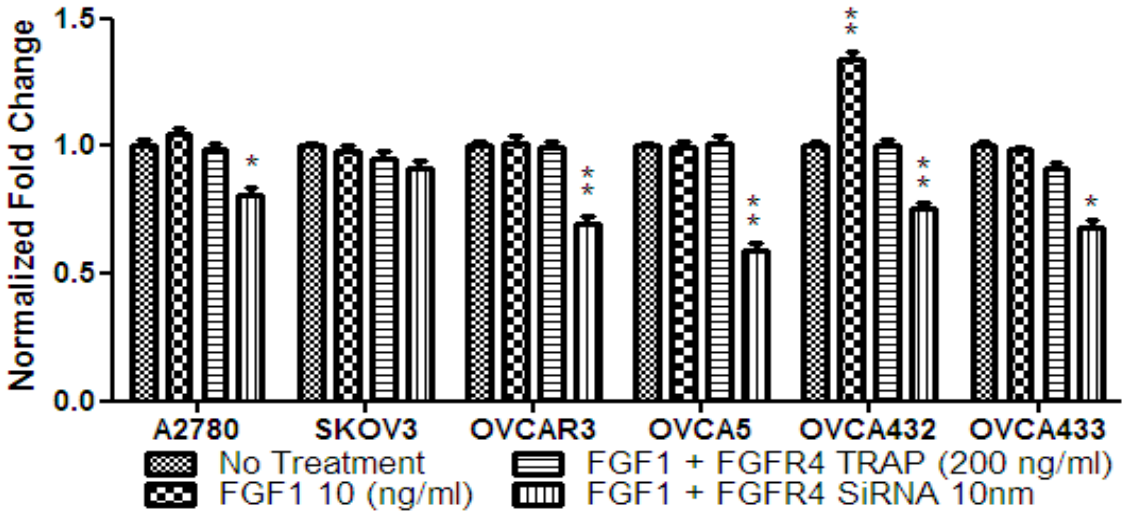


Figure R14. Effect of different inhibitors of the FGF pathway on HGSC in vitro(A)HGSC cell lines were plated and serum starved for 24 hours and then media was removed with medium containing 2% FBS and one of 3 FGF pathway inhibitors and difference in proliferation measured via WST-1 assay, no significant difference is noted(B)HGSC cell lines were plated and serum starved for 24 hours and then media was removed with medium containing 2% FBS and 10 ng/ml FGF1 and the FGFR4 pathway was either knocked down transiently or blocked via FGFR4 trap protein, decrease in proliferation below normalization point is noted in the FGFR4 knock down arm and FGFR4 trap successfully inhibited the effect of exogenous FGF1 on OVCA432 cell line *=p<0.05, **=p<0.01, ***=p<0.001)

FGFR4 knock down *in vitro* decreases cell survival, proliferation and migration

Transfection with *FGFR4* targeted siRNA resulted in significantly decreased cell survival (in serum reduced media) by 31-65% (Figure R15) and decreased proliferation in media supplemented with 10% FBS by greater than 50% in all cell lines tested (Figure R16) . Migration of several cell lines was also decreased significantly from control (Figure R16).

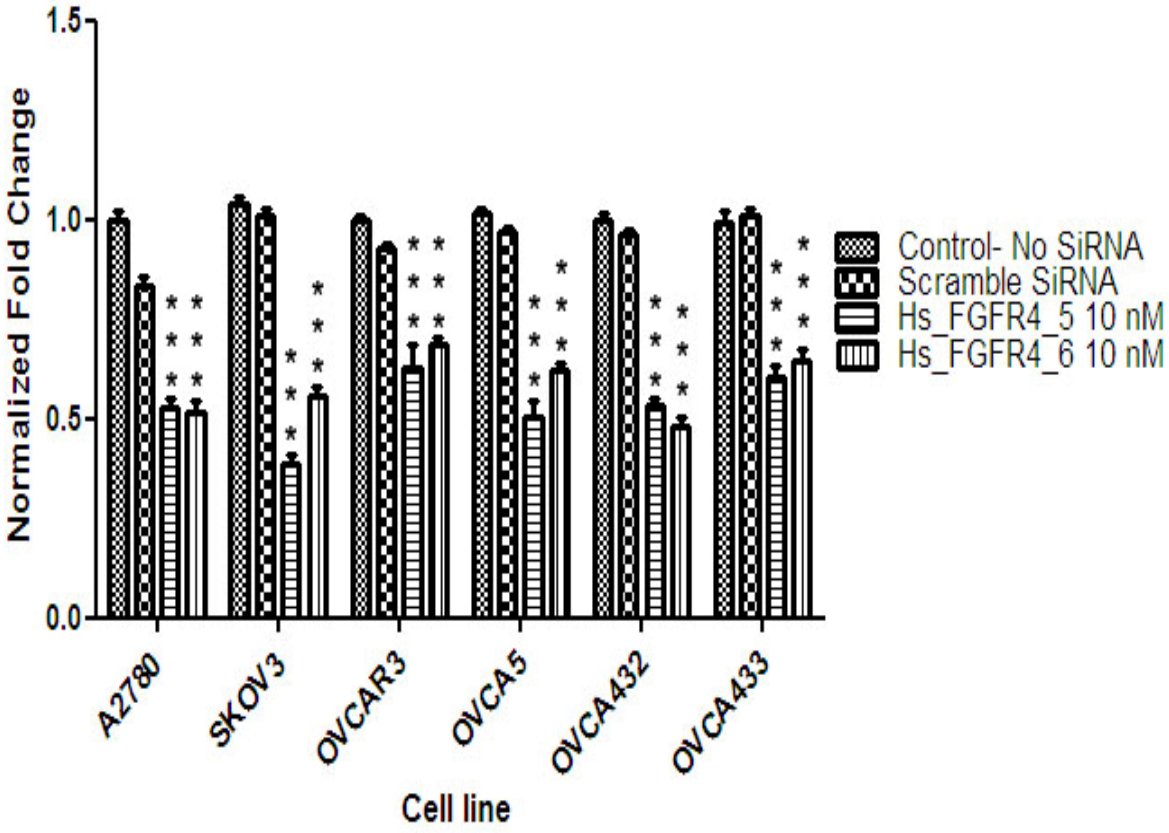


Figure R15. Effects of transient FGFR4 siRNA knockdown on HGSC Survival in vitro. Survival of HGSC cell lines after forward transfection with siRNA and incubation for 72 h in serum reduced media, significantly reduced survival was noted in cells transfected with *FGFR4* targeted siRNA compared to those with scramble siRNA as measured by WST-1 assays,***=p<0.001.

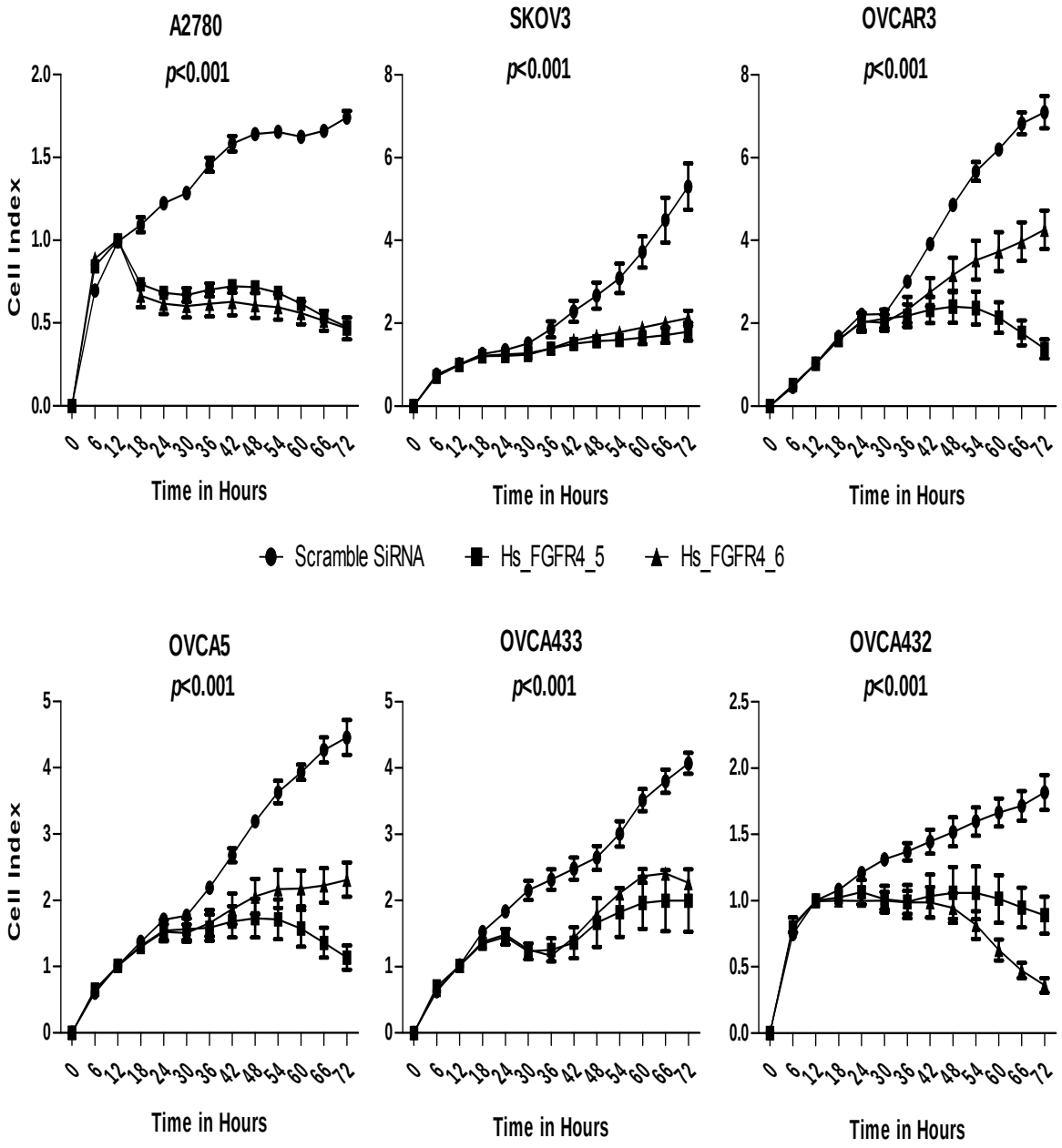
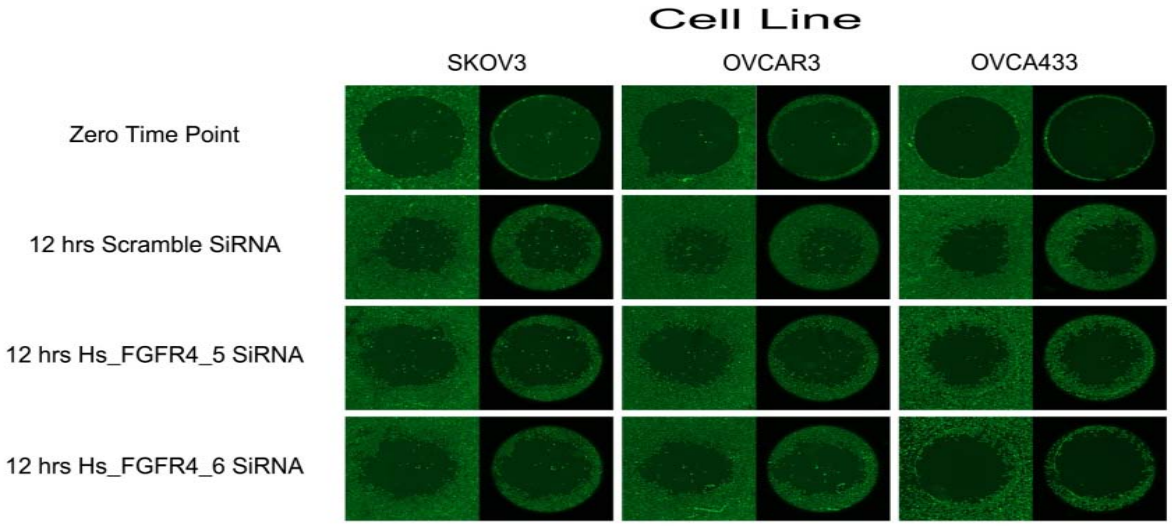


Figure R16. Effects of transient *FGFR4* siRNA knockdown on HGSC proliferation *in vitro.* Proliferation of HGSC cell lines after forward transfection with siRNA at multiple time points over 72 hr period, inhibition of proliferation was demonstrated by both *FGFR4* targeted siRNAs.

A



B

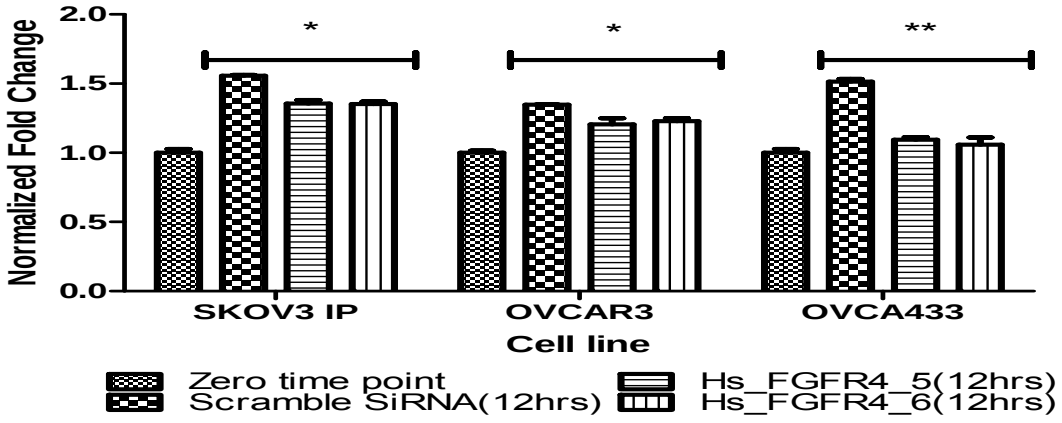


Figure R17. Effects of transient *FGFR4* siRNA knockdown on HGSC migration in vitro. (A) Migration/invasion of HGSC cell lines into collagen matrix using ORIS assay system with and without the assay mask; (B) Quantization of migration/invasion into the central zone of ORIS assay via measurement of green fluorescence demonstrating a significant decrease in migration after *FGFR4* knockdown(* = p<0.05 , **=p<0.01).

FGFR4 kinase and inter-membrane domains from clinical samples do not contain activating mutations

6 patients out of 43 (14%) were positive for the Gly388Arg polymorphism in the intermembrane domain. Otherwise sequencing of exons 9 through 16 did not demonstrate any mutations in either the kinase or intermembrane domain.

FGFR trap protein treatment reduced HGSC xenograft growth *in vivo*

The *FGFR1* trap protein was able to significantly reduce growth of xenografts OVCA432 in *vivo* as measured by serial luminescent imaging (Figure R18 & R19). H&E staining of xenografts revealed increased tumor necrosis and reduced tumor surrounding stroma (Figure 20)

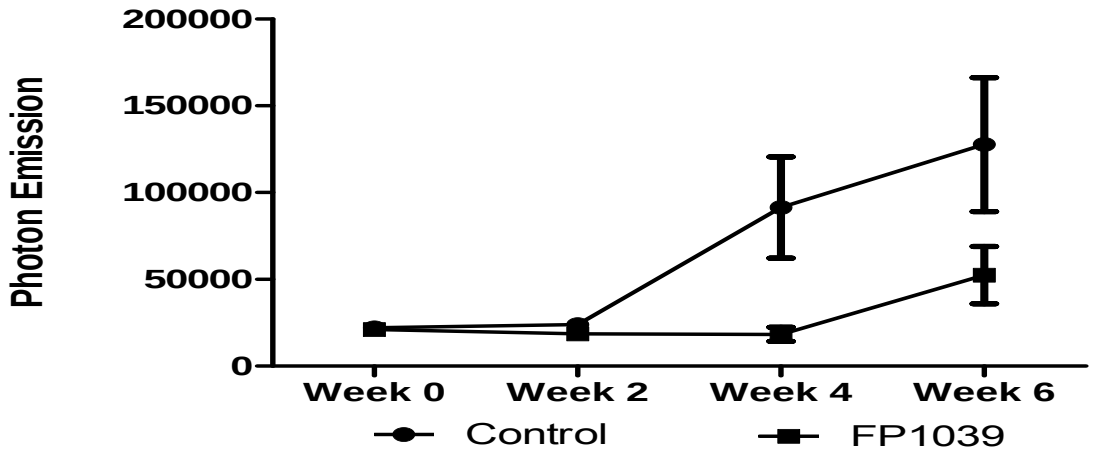


Figure R18. Quantitative Luminescent imaging of OVCA432 xenograft model treated with FP 1039. Two groups of mice, each injected IP with $1x10^6$ cells of OVCA432luc, then were imaged at baseline 1 week after injection (week 0). Control group was injected 20 mg pooled human IgG twice weekly and FP 1039 group was injected with the FGFR1 trap protein at the same dose and schedule. Significantly more luminescence is noted from the control tumor group $p<0.05$

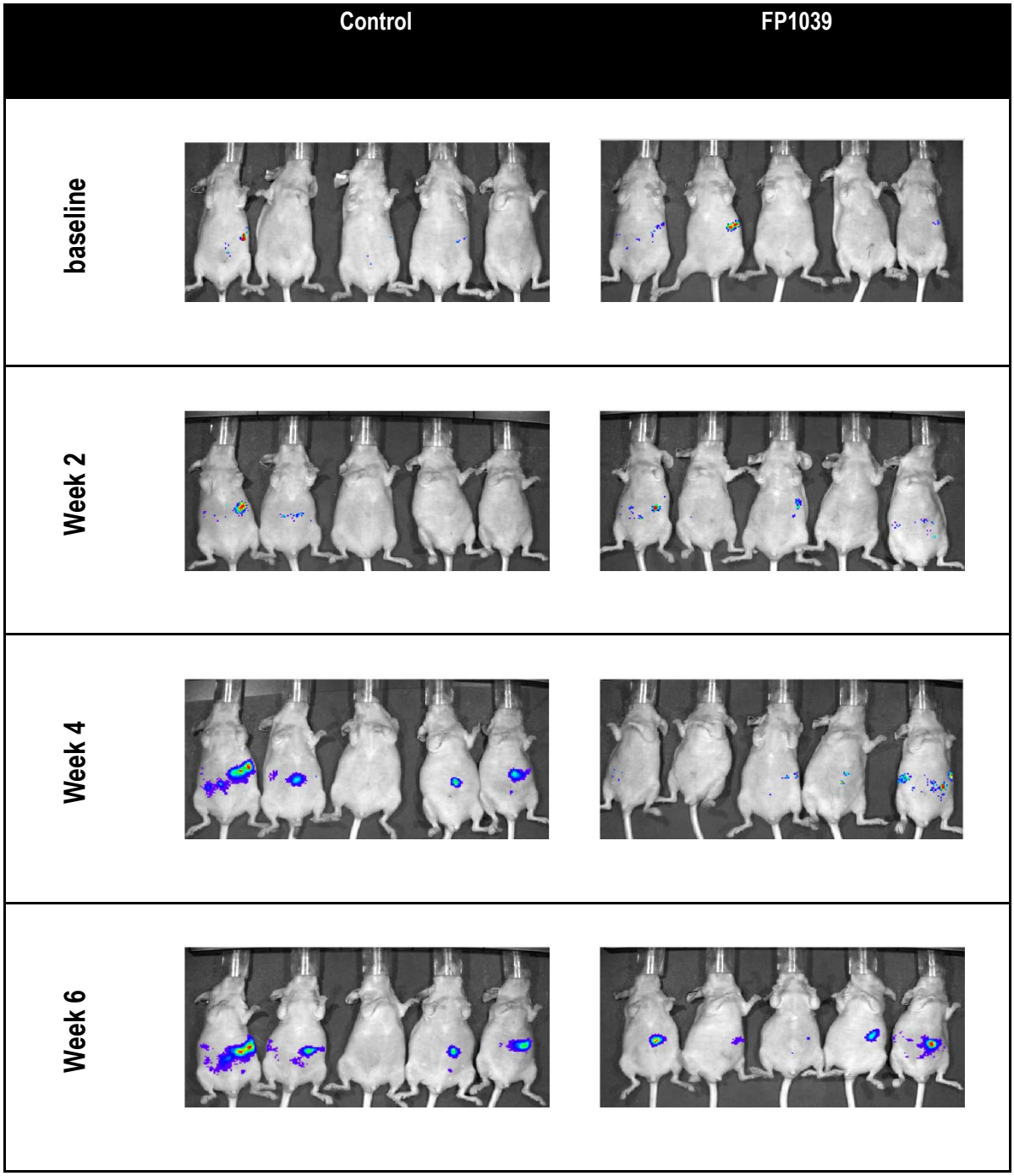


Figure R 19. Tumor luminescence and distribution in OVCA432 treated with FP1039 versus control. Inhibition of tumor growth is noted in the FP1039 group versus control.

A

B

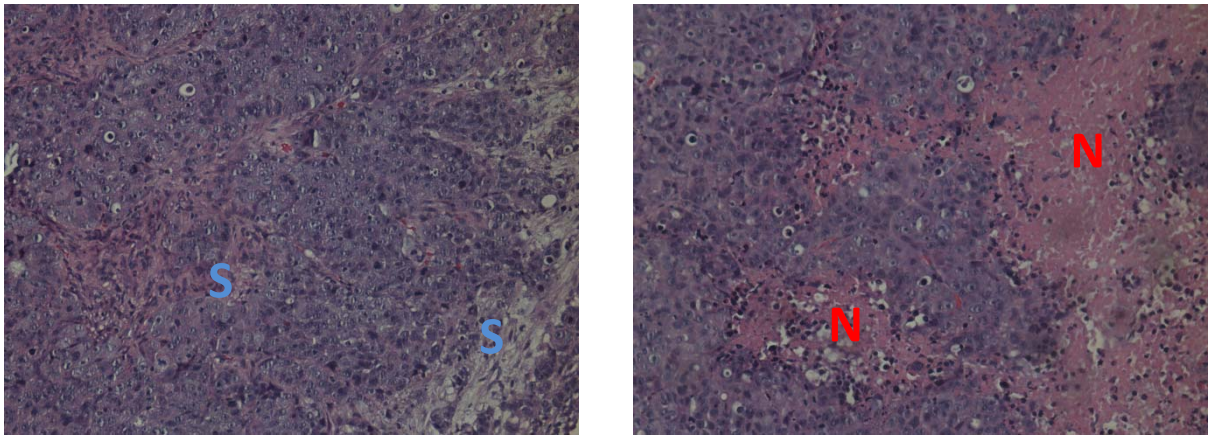


Figure 20. H&E sections from orthotopic xenograft tumors of OVCA432 in nude mice. (A) tumors from a mouse treated with pooled human IgG (B) Tumor from a mouse treated with FP 1039 showing increased necrosis (N)and minimal stroma compared to placebo group(S)

Discussion and Conclusions

Advanced stage high-grade serous carcinoma, as with many solid tumors, has undergone optimization of traditional therapeutic approaches over the last decade. Although this has led to improvements in survival and initial response to therapy, most patients with advanced stage disease still face poor prognosis, especially when in relapse or with metastatic disease[6, 7]. Characterization of genetic aberrations in these tumors and better understanding of molecular pathways involved in their pathogenesis/progression has fueled the development of targeted biologic agents for therapeutic intervention. When compared to traditional chemotherapy these agents posses a greater therapeutic index secondary to their relative selectivity for neoplastic cells[44]. In addition, biologic agents may have activity against those tumors that have become resistant to cytotoxic drugs. The heterogeneity of solid tumor genetic aberrations between patients has made it necessary to tailor such biologic therapy to the suitable molecular targets present within a tumor[79]. Our work demonstrates a possible target for such a personalized therapeutic approach in HGSC.

Our previous report demonstrated the negative impact on survival in HGSC tumors harboring amplification of segment 5q31-35.3[10]. Although that segment contains hundreds of genes, it is notable for presence of several members of the FGF family.

Evidence for the FGF pathway's involvement in tumor development and progression is substantial. Prior reports have demonstrated the role of the FGF family in driving cell proliferation in solid tumors, including prostate and endometrial carcinomas[42, 80]. The proliferative effects appear to be mediated principally through the MAPK

pathway[27]. Successful inhibition of FGF pathway mediated cancer growth with biologic agents has been reported in vitro [54].

The mitogenic effects of FGF signaling may also be enhanced by activation of antiapoptotic pathways through the PI3K pathway. In small cell lung cancer FGF2 overexpression is a known negative prognostic indicator. The decreased survival associated with FGF2 appears to be mediated by a cytoprotective effect involving upregulation of the expression of the antiapoptotic proteins Bcl2, Bcl-xL through S6 kinase[81].

FGF signaling can also promote cell migration. Pancreatic cancer cells in vitro, have shown FGF10 and FGFR2 dependent invasion[82]. In a breast cancer mouse model activation of FGFR1 kinase domain led to invasive mammary lesions. In that model FGFR1 activation induced cell proliferation and survival, in addition to the gain of a matrix metalloproteinase 3, resulting in an invasive phenotype[83].

Our results demonstrate that activation of the FGF signaling axis through FGFR4 in HGSC leads to downstream activation of similar critical signaling pathways associated with phenotypic changes characteristic of more aggressive carcinomas.

We have demonstrated the activation of MAPK pathway by *FGF1/FGFR4* interaction and resulting proliferative effect. Overexpression of the FGF1 ligand and the FGFR4 receptor in tumors with the 5q31-35.3 therefore may provide an autocrine signaling loop that drives tumor growth and proliferation in HGSC. Through the same loop the PI3K pathway may decrease apoptosis and increased survival of tumor cells. Additional FGF ligand involvement is likely as knock down of *FGFR4* in absence of FGF1 and without known activating mutations leads to decreased cell proliferation and

cell death. This may be due to autocrine *FGF18* overexpression, as it is also located on 5q311-35.3 amplicon, or other autocrine/paracrine FGF ligand production. It is also evident that other pathways including WNT and NFkB are involved and their role is currently under investigation by our group.

Our RPPA results also support that FGF1's effect on cancer cells is mediated by activation of several signaling pathways. It also suggests that FGF1 signaling may play a more direct role in inhibiting apoptosis secondary to up regulation of Bcl-xL, and migration/invasion as evidenced by downregulation of E-Cadherin. This is consistent with previously published reports discussed earlier. However, the evidence we present is preliminary and requires further investigation.

It also appears that different levels of *FGFR4* amplification may have different effects on cell response in HGSC. The response to exogenous FGF1 by OVCA432, the highest expressor of *FGFR4,* demonstrated increased proliferation and survival but no change in migratory behaviour Other cell lines with lower FGFR4 expression demonstrated significant increases in migration/invasion in addition to survival but no increase in proliferation.

We did not observe *in vitro* production of FGF1 in conditioned media, although it was detected in cell lysate, and in tissue sections in our previous report [10]. This may be due to a short half life for the protein. It may also be that release *in* vivo requires certain conditions or co-signals that cannot be replicated by in monolayer cell culture environment.

We have shown that in an orthotopic mouse model of HGSC use of the FP-1039 FGFR1 trap protein significantly decreases tumor growth. FP-1039 is a fusion protein

of the extracellular domain of *FGFR1* and IgG Fc fragment. The trap protein has broad spectrum binding activity to FGF ligands including FGF1 and is currently in phase 2 trials in other gynecologic malignancies. The ability to sequester several FGF ligands and to inhibit the pathway upstream of the receptor allows FP-1039 to inhibit all the possible effects of overexpression of FGF ligands. This provides a therapeutic approach that interrupts autocrine signaling loops, hypothetically decreasing proliferation, survival and migration of tumor cells. Paracrine effects such as angiogenesis may also be inhibited.

The challenge remains in identifying patients that may benefit from such an approach. This may be possible through identifying genetic and epigenetic changes particularly in genes involved in the *FGF-FGFR* axis that are associated with the responsiveness of the FGFR trap in preclinical mouse models.

References

1. Jemal, A., R. Siegel, E. Ward, Y. Hao, J. Xu, and M.J. Thun, *Cancer statistics, 2009.* CA Cancer J Clin, 2009. **59**(4): p. 225-49.
2. Thigpen, J.T., R.B. Vance, and T. Khansur, *Second-line chemotherapy for recurrent carcinoma of the ovary.* Cancer, 1993. **71**(4 Suppl): p. 1559-64.
3. Cho, K.R. and M. Shih Ie, *Ovarian cancer.* Annu Rev Pathol, 2009. **4**: p. 287-313.
4. Cho, K.R., *Ovarian cancer update: lessons from morphology, molecules, and mice.* Arch Pathol Lab Med, 2009. **133**(11): p. 1775-81.
5. *NIH consensus conference. Ovarian cancer. Screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer.* JAMA, 1995. **273**(6): p. 491-7.
6. McGuire, W.P., W.J. Hoskins, M.F. Brady, P.R. Kucera, E.E. Partridge, K.Y. Look, D.L. Clarke-Pearson, and M. Davidson, *Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer.* N Engl J Med, 1996. **334**(1): p. 1-6.
7. Markman, M., R. Rothman, T. Hakes, B. Reichman, W. Hoskins, S. Rubin, W. Jones, L. Almadrones, and J.L. Lewis, Jr., *Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin.* J Clin Oncol, 1991. **9**(3): p. 389-93.
8. Yap, T.A., C.P. Carden, and S.B. Kaye, *Beyond chemotherapy: targeted therapies in ovarian cancer.* Nat Rev Cancer, 2009. **9**(3): p. 167-81.
9. Burger, R.A., *Overview of anti-angiogenic agents in development for ovarian cancer.* Gynecol Oncol. **121**(1): p. 230-8.

10. Birrer, M.J., M.E. Johnson, K. Hao, K.K. Wong, D.C. Park, A. Bell, W.R. Welch, R.S. Berkowitz, and S.C. Mok, *Whole genome oligonucleotide-based array comparative genomic hybridization analysis identified fibroblast growth factor 1 as a prognostic marker for advanced-stage serous ovarian adenocarcinomas.* J Clin Oncol, 2007. **25**(16): p. 2281-7.
11. Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun, M. Lathrop, G. Gyapay, J. Morissette, and J. Weissenbach, *A comprehensive genetic map of the human genome based on 5,264 microsatellites.* Nature, 1996. **380**(6570): p. 152-4.
12. Itoh, N. and D.M. Ornitz, *Evolution of the Fgf and Fgfr gene families.* Trends Genet, 2004. **20**(11): p. 563-9.
13. Wiedlocha, A. and V. Sorensen, *Signaling, internalization, and intracellular activity of fibroblast growth factor.* Curr Top Microbiol Immunol, 2004. **286**: p. 45-79.
14. Presta, M., P. Dell'Era, S. Mitola, E. Moroni, R. Ronca, and M. Rusnati, *Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis.* Cytokine & Growth Factor Reviews, 2005. **16**(2): p. 159-178.
15. Villegas, S.N., M. Canham, and J.M. Brickman, *FGF signalling as a mediator of lineage transitions--evidence from embryonic stem cell differentiation.* J Cell Biochem. **110**(1): p. 10-20.
16. Grose, R. and C. Dickson, *Fibroblast growth factor signaling in tumorigenesis.* Cytokine Growth Factor Rev, 2005. **16**(2): p. 179-86.
17. Beenken, A. and M. Mohammadi, *The FGF family: biology, pathophysiology and therapy.* Nat Rev Drug Discov, 2009. **8**(3): p. 235-253.

18. Turner, N. and R. Grose, *Fibroblast growth factor signalling: from development to cancer.* Nat Rev Cancer, 2010. **10**(2): p. 116-129.

19. Zhong, C., G. Saribekyan, C.P. Liao, M.B. Cohen, and P. Roy-Burman, *Cooperation between FGF8b overexpression and PTEN deficiency in prostate tumorigenesis.* Cancer Res, 2006. **66**(4): p. 2188-94.

20. Song, S., M.G. Wientjes, Y. Gan, and J.L. Au, *Fibroblast growth factors: an epigenetic mechanism of broad spectrum resistance to anticancer drugs.* Proc Natl Acad Sci U S A, 2000. **97**(15): p. 8658-63.

21. Nomura, S., H. Yoshitomi, S. Takano, T. Shida, S. Kobayashi, M. Ohtsuka, F. Kimura, H. Shimizu, H. Yoshidome, A. Kato, and M. Miyazaki, *FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer.* Br J Cancer, 2008. **99**(2): p. 305-13.

22. Savagner, P., K.M. Yamada, and J.P. Thiery, *The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition.* J Cell Biol, 1997. **137**(6): p. 1403-19.

23. Wang, Y. and D. Becker, *Antisense targeting of basic fibroblast growth factor and fibroblast growth factor receptor-1 in human melanomas blocks intratumoral angiogenesis and tumor growth.* Nat Med, 1997. **3**(8): p. 887-93.

24. Singer, G., R. Stohr, L. Cope, R. Dehari, A. Hartmann, D.F. Cao, T.L. Wang, R.J. Kurman, and M. Shih Ie, *Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation.* Am J Surg Pathol, 2005. **29**(2): p. 218-24.

25. Powers, C., S. McLeskey, and A. Wellstein, *Fibroblast growth factors, their receptors and signaling.* Endocr Relat Cancer, 2000. **7**(3): p. 165-197.

26. Eswarakumar, V.P., I. Lax, and J. Schlessinger, *Cellular signaling by fibroblast growth factor receptors.* Cytokine Growth Factor Rev, 2005. **16**(2): p. 139-49.

27. Dailey, L., D. Ambrosetti, A. Mansukhani, and C. Basilico, *Mechanisms underlying differential responses to FGF signaling.* Cytokine Growth Factor Rev, 2005. **16**(2): p. 233-47.

28. Plotnikov, A.N., J. Schlessinger, S.R. Hubbard, and M. Mohammadi, *Structural basis for FGF receptor dimerization and activation.* Cell, 1999. **98**(5): p. 641-50.

29. Furdui, C.M., E.D. Lew, J. Schlessinger, and K.S. Anderson, *Autophosphorylation of FGFR1 kinase is mediated by a sequential and precisely ordered reaction.* Mol Cell, 2006. **21**(5): p. 711-7.

30. Gotoh, N., *Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins.* Cancer Sci, 2008. **99**(7): p. 1319-25.

31. Ong, S.H., Y.R. Hadari, N. Gotoh, G.R. Guy, J. Schlessinger, and I. Lax, *Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins.* Proc Natl Acad Sci U S A, 2001. **98**(11): p. 6074-9.

32. Peters, K.G., J. Marie, E. Wilson, H.E. Ives, J. Escobedo, M. Del Rosario, D. Mirda, and L.T. Williams, *Point mutation of an FGF receptor abolishes phosphatidylinositol turnover and Ca2+ flux but not mitogenesis.* Nature, 1992. **358**(6388): p. 678-81.

33. Hart, K.C., S.C. Robertson, M.Y. Kanemitsu, A.N. Meyer, J.A. Tynan, and D.J. Donoghue, *Transformation and Stat activation by derivatives of FGFR1, FGFR3, and FGFR4.* Oncogene, 2000. **19**(29): p. 3309-20.

34. Cabrita, M.A. and G. Christofori, *Sprouty proteins, masterminds of receptor tyrosine kinase signaling.* Angiogenesis, 2008. **11**(1): p. 53-62.

35. Tsang, M. and I.B. Dawid, *Promotion and attenuation of FGF signaling through the Ras-MAPK pathway.* Sci STKE, 2004. **2004**(228): p. pe17.

36. Tsang, M., R. Friesel, T. Kudoh, and I.B. Dawid, *Identification of Sef, a novel modulator of FGF signalling.* Nat Cell Biol, 2002. **4**(2): p. 165-9.

37. Kovalenko, D., X. Yang, R.J. Nadeau, L.K. Harkins, and R. Friesel, *Sef inhibits fibroblast growth factor signaling by inhibiting FGFR1 tyrosine phosphorylation and subsequent ERK activation.* J Biol Chem, 2003. **278**(16): p. 14087-91.

38. Bronner, F., M.C. Farach-Carson, H.I. Roach, and P.J. Marie, *FGF/FGFR Signaling in Skeletal Dysplasias*, in *Bone and Development*, Springer London. p. 93-105.

39. Chesi, M., L.A. Brents, S.A. Ely, C. Bais, D.F. Robbiani, E.A. Mesri, W.M. Kuehl, and P.L. Bergsagel, *Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma.* Blood, 2001. **97**(3): p. 729-36.

40. Knights, V. and S.J. Cook, *De-regulated FGF receptors as therapeutic targets in cancer.* Pharmacology & Therapeutics, 2009. **125**(1): p. 105-117.

41. Munro, N.P. and M.A. Knowles, *Fibroblast growth factors and their receptors in transitional cell carcinoma.* J. Urol., 2003. **169**: p. 675-682.

42. Dutt, A., *Drug-sensitive FGFR2 mutations in endometrial carcinoma.* Proc. Natl Acad. Sci. USA, 2008. **105**: p. 8713-8717.

43. Reis-Filho, J.S., P.T. Simpson, N.C. Turner, M.B. Lambros, C. Jones, A. Mackay, A. Grigoriadis, D. Sarrio, K. Savage, T. Dexter, M. Iravani, K. Fenwick, B. Weber, D. Hardisson, F.C. Schmitt, J. Palacios, S.R. Lakhani, and A. Ashworth, *FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas.* Clin Cancer Res, 2006. **12**(22): p. 6652-62.

44. Berz, D. and H. Wanebo, *Targeting the growth factors and angiogenesis pathways: Small molecules in solid tumors.* J Surg Oncol. **103**(6): p. 574-86.

45. Hunter, D.J., *A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer.* Nature Genet., 2007. **39**: p. 870-874.

46. Courjal, F., M. Cuny, J. Simony-Lafontaine, G. Louason, P. Speiser, R. Zeillinger, C. Rodriguez, and C. Theillet, *Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: definition of phenotypic groups.* Cancer Res, 1997. **57**(19): p. 4360-7.

47. Jacquemier, J., J. Adelaide, P. Parc, F. Penault-Llorca, J. Planche, O. deLapeyriere, and D. Birnbaum, *Expression of the FGFR1 gene in human breast-carcinoma cells.* Int J Cancer, 1994. **59**(3): p. 373-8.

48. Gorringe, K.L., S. Jacobs, E.R. Thompson, A. Sridhar, W. Qiu, D.Y. Choong, and I.G. Campbell, *High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications.* Clin Cancer Res, 2007. **13**(16): p. 4731-9.

49. Simon, R., J. Richter, U. Wagner, A. Fijan, J. Bruderer, U. Schmid, D. Ackermann, R. Maurer, G. Alund, H. Knonagel, M. Rist, K. Wilber, M. Anabitarte, F. Hering, T. Hardmeier, A. Schonenberger, R. Flury, P. Jager, J.L. Fehr, P. Schraml, H. Moch, M.J. Mihatsch, T. Gasser, and G. Sauter, *High-throughput tissue microarray analysis of 3p25 (RAF1) and 8p12 (FGFR1) copy number alterations in urinary bladder cancer.* Cancer Res, 2001. **61**(11): p. 4514-9.

50. Missiaglia, E., J. Selfe, M. Hamdi, D. Williamson, G. Schaaf, C. Fang, J. Koster, B. Summersgill, B. Messahel, R. Versteeg, K. Pritchard-Jones, M. Kool, and J. Shipley, *Genomic imbalances in rhabdomyosarcoma cell lines affect expression of genes frequently altered in primary tumors: an approach to identify candidate genes involved in tumor development.* Genes Chromosomes Cancer, 2009. **48**(6): p. 455-67.

51. Lin, W.M., A.C. Baker, R. Beroukhim, W. Winckler, W. Feng, J.M. Marmion, E. Laine, H. Greulich, H. Tseng, C. Gates, F.S. Hodi, G. Dranoff, W.R. Sellers, R.K. Thomas, M. Meyerson, T.R. Golub, R. Dummer, M. Herlyn, G. Getz, and L.A. Garraway, *Modeling genomic diversity and tumor dependency in malignant melanoma.* Cancer Res, 2008. **68**(3): p. 664-73.

52. Kunii, K., L. Davis, J. Gorenstein, H. Hatch, M. Yashiro, A. Di Bacco, C. Elbi, and B. Lutterbach, *FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival.* Cancer Res, 2008. **68**(7): p. 2340-8.

53. Takeda, M., T. Arao, H. Yokote, T. Komatsu, K. Yanagihara, H. Sasaki, Y. Yamada, T. Tamura, K. Fukuoka, H. Kimura, N. Saijo, and K. Nishio, *AZD2171*

shows potent antitumor activity against gastric cancer over-expressing fibroblast growth factor receptor 2/keratinocyte growth factor receptor. Clin Cancer Res, 2007. **13**(10): p. 3051-7.

54. Dutt, A., H.B. Salvesen, T.H. Chen, A.H. Ramos, R.C. Onofrio, C. Hatton, R. Nicoletti, W. Winckler, R. Grewal, M. Hanna, N. Wyhs, L. Ziaugra, D.J. Richter, J. Trovik, I.B. Engelsen, I.M. Stefansson, T. Fennell, K. Cibulskis, M.C. Zody, L.A. Akslen, S. Gabriel, K.K. Wong, W.R. Sellers, M. Meyerson, and H. Greulich, *Drug-sensitive FGFR2 mutations in endometrial carcinoma.* Proc Natl Acad Sci U S A, 2008. **105**(25): p. 8713-7.

55. Jang, J.H., K.H. Shin, and J.G. Park, *Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers.* Cancer Res, 2001. **61**(9): p. 3541-3.

56. Easton, D.F., K.A. Pooley, A.M. Dunning, P.D. Pharoah, D. Thompson, D.G. Ballinger, J.P. Struewing, J. Morrison, H. Field, R. Luben, N. Wareham, S. Ahmed, C.S. Healey, R. Bowman, K.B. Meyer, C.A. Haiman, L.K. Kolonel, B.E. Henderson, L. Le Marchand, P. Brennan, S. Sangrajrang, V. Gaborieau, F. Odefrey, C.Y. Shen, P.E. Wu, H.C. Wang, D. Eccles, D.G. Evans, J. Peto, O. Fletcher, N. Johnson, S. Seal, M.R. Stratton, N. Rahman, G. Chenevix-Trench, S.E. Bojesen, B.G. Nordestgaard, C.K. Axelsson, M. Garcia-Closas, L. Brinton, S. Chanock, J. Lissowska, B. Peplonska, H. Nevanlinna, R. Fagerholm, H. Eerola, D. Kang, K.Y. Yoo, D.Y. Noh, S.H. Ahn, D.J. Hunter, S.E. Hankinson, D.G. Cox, P. Hall, S. Wedren, J. Liu, Y.L. Low, N. Bogdanova, P. Schurmann, T. Dork, R.A. Tollenaar, C.E. Jacobi, P. Devilee, J.G. Klijn, A.J. Sigurdson, M.M. Doody, B.H. Alexander, J. Zhang, A. Cox, I.W. Brock, G. MacPherson,

M.W. Reed, F.J. Couch, E.L. Goode, J.E. Olson, H. Meijers-Heijboer, A. van den Ouweland, A. Uitterlinden, F. Rivadeneira, R.L. Milne, G. Ribas, A. Gonzalez-Neira, J. Benitez, J.L. Hopper, M. McCredie, M. Southey, G.G. Giles, C. Schroen, C. Justenhoven, H. Brauch, U. Hamann, Y.D. Ko, A.B. Spurdle, J. Beesley, X. Chen, A. Mannermaa, V.M. Kosma, V. Kataja, J. Hartikainen, N.E. Day, D.R. Cox and B.A. Ponder, *Genome-wide association study identifies novel breast cancer susceptibility loci.* Nature, 2007. **447**(7148): p. 1087-93.

57. Hunter, D.J., P. Kraft, K.B. Jacobs, D.G. Cox, M. Yeager, S.E. Hankinson, S. Wacholder, Z. Wang, R. Welch, A. Hutchinson, J. Wang, K. Yu, N. Chatterjee, N. Orr, W.C. Willett, G.A. Colditz, R.G. Ziegler, C.D. Berg, S.S. Buys, C.A. McCarty, H.S. Feigelson, E.E. Calle, M.J. Thun, R.B. Hayes, M. Tucker, D.S. Gerhard, J.F. Fraumeni, R.N. Hoover, G. Thomas, and S.J. Chanock, *A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer.* Nat Genet, 2007. **39**(7): p. 870-874.

58. Nord, H., U. Segersten, J. Sandgren, K. Wester, C. Busch, U. Menzel, J. Komorowski, J.P. Dumanski, P.-U. Malmström, and T.D. de Ståhl, *Focal amplifications are associated with high grade and recurrences in stage Ta bladder carcinoma.* International Journal of Cancer. **126**(6): p. 1390-1402.

59. Vekony, H., B. Ylstra, S.M. Wilting, G.A. Meijer, M.A. van de Wiel, C.R. Leemans, I. van der Waal, and E. Bloemena, *DNA copy number gains at loci of growth factors and their receptors in salivary gland adenoid cystic carcinoma.* Clin Cancer Res, 2007. **13**(11): p. 3133-9.

60. Cappellen, D., *Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas.* Nature Genet., 1999. **23**: p. 18-20.

61. Rosty, C., *Clinical and biological characteristics of cervical neoplasias with FGFR3 mutation.* Mol. Cancer, 2005. **4**: p. 15.

62. Hernandez, S., *FGFR3 mutations in prostate cancer: association with low-grade tumors.* Mod. Pathol., 2009. **22**: p. 848-856.

63. Goriely, A., *Activating mutations in FGFR3 and HRAS reveal a shared genetic origin for congenital disorders and testicular tumors.* Nature Genet., 2009. **41**: p. 1247-1252.

64. Onwuazor, O.N., *Mutation, SNP, and isoform analysis of fibroblast growth factor receptor 3 (FGFR3) in 150 newly diagnosed multiple myeloma patients.* Blood, 2003. **102**: p. 772-773.

65. Taylor, J.G.t., A.T. Cheuk, P.S. Tsang, J.Y. Chung, Y.K. Song, K. Desai, Y. Yu, Q.R. Chen, K. Shah, V. Youngblood, J. Fang, S.Y. Kim, C. Yeung, L.J. Helman, A. Mendoza, V. Ngo, L.M. Staudt, J.S. Wei, C. Khanna, D. Catchpoole, S.J. Qualman, S.M. Hewitt, G. Merlino, S.J. Chanock, and J. Khan, *Identification of FGFR4-activating mutations in human rhabdomyosarcomas that promote metastasis in xenotransplanted models.* J Clin Invest, 2009. **119**(11): p. 3395-407.

66. Bange, J., *Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele.* Cancer Res., 2002. **62**: p. 840-847.

67. Spinola, M., *FGFR4 Gly388Arg polymorphism and prognosis of breast and colorectal cancer.* Oncol. Rep., 2005. **14**: p. 415-419.

68. Kunii, K., *FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival.* Cancer Res., 2008. **68**: p. 2340-2348.

69. Landen, C.N., T.J. Kim, Y.G. Lin, W.M. Merritt, A.A. Kamat, L.Y. Han, W.A. Spannuth, A.M. Nick, N.B. Jennnings, M.S. Kinch, D. Tice, and A.K. Sood, *Tumor-selective response to antibody-mediated targeting of alphavbeta3 integrin in ovarian cancer.* Neoplasia, 2008. **10**(11): p. 1259-67.

70. Buick, R.N., R. Pullano, and J.M. Trent, *Comparative properties of five human ovarian adenocarcinoma cell lines.* Cancer Res, 1985. **45**(8): p. 3668-76.

71. Yoneda, J., H. Kuniyasu, M.A. Crispens, J.E. Price, C.D. Bucana, and I.J. Fidler, *Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice.* J Natl Cancer Inst, 1998. **90**(6): p. 447-54.

72. Bast, R.C., Jr., M. Feeney, H. Lazarus, L.M. Nadler, R.B. Colvin, and R.C. Knapp, *Reactivity of a monoclonal antibody with human ovarian carcinoma.* J Clin Invest, 1981. **68**(5): p. 1331-7.

73. Lau, D.H., A.D. Lewis, M.N. Ehsan, and B.I. Sikic, *Multifactorial mechanisms associated with broad cross-resistance of ovarian carcinoma cells selected by cyanomorpholino doxorubicin.* Cancer Res, 1991. **51**(19): p. 5181-7.

74. Vistejnova, L., J. Dvorakova, M. Hasova, T. Muthny, V. Velebny, K. Soucek, and L. Kubala, *The comparison of impedance-based method of cell proliferation monitoring with commonly used metabolic-based techniques.* Neuro Endocrinol Lett, 2009. **30 Suppl 1**: p. 121-7.

75. Ungefroren, H., S. Sebens, S. Groth, F. Gieseler, and F. Fandrich, *Differential roles of Src in transforming growth factor-ss regulation of growth arrest, epithelial-to-mesenchymal transition and cell migration in pancreatic ductal adenocarcinoma cells.* Int J Oncol. **38**(3): p. 797-805.

76. Keogh, R.J., *New technology for investigating trophoblast function.* Placenta. **31**(4): p. 347-50.

77. Park, E.S., R. Rabinovsky, M. Carey, B.T. Hennessy, R. Agarwal, W. Liu, Z. Ju, W. Deng, Y. Lu, H.G. Woo, S.B. Kim, J.H. Cheong, L.A. Garraway, J.N. Weinstein, G.B. Mills, J.S. Lee, and M.A. Davies, *Integrative analysis of proteomic signatures, mutations, and drug responsiveness in the NCI 60 cancer cell line set.* Mol Cancer Ther. **9**(2): p. 257-67.

78. Tibes, R., Y. Qiu, Y. Lu, B. Hennessy, M. Andreeff, G.B. Mills, and S.M. Kornblau, *Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells.* Molecular Cancer Therapeutics, 2006. **5**(10): p. 2512-2521.

79. Solyanik, G.I., *Multifactorial nature of tumor drug resistance.* Exp Oncol. **32**(3): p. 181-5.

80. Kwabi-Addo, B., M. Ozen, and M. Ittmann, *The role of fibroblast growth factors and their receptors in prostate cancer.* Endocr. Relat. Cancer, 2004. **11**: p. 709-724.

81. Pardo, O.E., *FGF-2 protects small cell lung cancer cells from apoptosis through a complex involving PKCepsilon, B-Raf and S6K2.* EMBO J., 2006. **25**: p. 3078-3088.

82. Nomura, S., *FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer.* Br. J. Cancer, 2008. **99**: p. 305-313.

83. Freeman, K.W., *Inducible prostate intraepithelial neoplasia with reversible hyperplasia in conditional FGFR1-expressing mice.* Cancer Res., 2003. **63**: p. 8256-8263.

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