GENE EXPRESSION ANALYSIS OF FIBROBLAST GROWTH FACTOR IN A RAT MODEL OF HUMAN ENDOMETRIAL CANCER

MASTER DEGREE PROJECT IN BIOMEDICINE
BM701A, 30 ECTS

AUTHOR:
Vipul Chakole
g13vipch@student.his.se
vchakole86@gmail.com

Supervisor:
Neha Singh
neha.singh@his.se

Examiner:
Sandra Karlsson
Sandra.karlsson@his.se

School of life sciences.
University of Skövde.
ABSTRACT

The fibroblast growth factor receptor (FGF/FGFR) signaling has a significant role in normal organ development, like vascular and skeletal development. The dysregulation of the fibroblast growth receptor signaling occurs due to genetic modification or over expression of the receptor. This has been observed in different carcinomas [34,35]. The endometrial carcinoma is the most common gynecologic malignancy in western counties and fourth most common cancer among women worldwide. Type I endometrial carcinomas constitute approximately 70 to 80% of all endometrium cancer. It follows estrogens related pathways in carcinogenesis. The BDII rat model is an ideal model for hormonal carcinogenesis because 90% of the female virgin spontaneously develop type I hormone independent endometrial adenocarcinomas within two years of age. Fibroblast growth factor (FGF) ligands via FGFR combined with heparan sulfate proteoglycans (HPSG) in extracellular matrix with the help of proteases and participate in the signal transmission of various functions such as cell proliferation, cell survival, cell motility. FGF signaling pathway is found to be aberrantly expressed in many of the cancers such as prostate and breast carcinomas. The main aim of the study was to investigate the differential expression of FGF gene in the malignant and non-malignant cell lines of BDII rat endometrial strains along with human embryonic kidney cell & Ishikawa cell lines [1, 2, 34, 35, 36]. Real-time qPCR was used for analyzing relative expression of FGF gene between malignant and non-malignant cell lines in this study. The result showed a higher trend of the FGF gene in the non-malignant cell lines, compared to the expression in the malignant cell lines. It can be conspicuously seen that the FGF gene has shown higher trend in the Ishikawa cell line but cannot conclude about significantly over expression of FGF gene in this study due to very low number of samples. Moreover, this study should confirm by using additional techniques such as Western blot, Immunofluorescence to check the expression of FGF at protein level and cellular level.
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# LIST OF ABBREVIATION
BDII          BDII/Han (inbred rat strain)
BN            Brown- Norway
SPRD-Cu 3     Sprague-Dawley-curly3 inbred rat strain
cDNA          complementary Deoxyribo nucleic Acid
RNA           Ribonucleic acid
DNA           Deoxyribo Nucleic Acid
FGF           Fibroblast growth factor
EC            Endometrial cancer
EAC           Endometrial adenocarcinoma
ERK           extracellular signal-related kinase
GF            growth factor
SPRY          sprouty proteins
HPSG          heparan sulfate proteoglycans
HEK           Human embryonic kidney cell
PLC           phospholipase C
NUT           rat uterine tumour developed in the backcross (N1) progeny
NME           Non-malignant Endometrium
RT-PCR        Reverse transcriptase polymerase chain reaction
qPCR          Real time quantitative PCR
SOS           son of sevenless
GRB2          protein Growth factor receptor bound 2
GRB1          associated binding protein 1
FRS2          Intracellular receptor substrates 2
MAPK          mitogen-activated protein kinase
PI3K          Ras independent phosphoinositide 3 kinase
RPMI          Roswell, Park Memorial Institute

1. INTRODUCTION
**Endometrial Carcinoma**

Endometrial carcinoma (EC) is the most common gynecologic malignancy in western counties and fourth most common cancer among women worldwide. Almost all cancer of uterus starts in endometrium layer of uterus, and these are called endometrial carcinomas. In addition to endometrium, cancer can also develop in the muscular layer which supports connective tissue of uterus and these are called sarcomas. The endometrial carcinomas developed in those cells which form glands in the endometrium is called endometrial adenocarcinomas (EAC). EC can be divided into two categories on morphology basis: Type 1 is endometrial carcinoma in which approximately 70 to 80% of all endometrium cancer follows estrogens related pathways, and type 2 is non-endometrial carcinomas which arise from endometrial intra-epithelial carcinoma. EAC is mostly common type 1 as it originates from glandular cells of uterus epithelium surface. Type 2 occur post-menopausal and type 1 occur pre-menopausal in women. Tumors are cancerous cells forming gland and this could be due to excess of estrogen hormone in the endometrium cell lining. The high levels of estrogen hormone make the cell lining look cancerous as they grow and spread outside the uterus. The endometrium tissue is dependent on hormone and excess administration of estrogen is one of the factors that cause EAC [2, 3].

**Animal model**

EAC is a complex disease and studies on the carcinogenesis are difficult to perform on human tumor samples. This is because of high genetic heterogeneity in humans and the influence of environmental factor. Model organisms, like inbred rat strains, are common in experiments because it has similarity in histopathological and pathogenesis property with humans. Hence, inbred rat models like BDII/han are commonly used for the study of endometrium adenocarcinoma. It was first described in 1987 that 90% of female or even more BDII/Han virgins quickly developed EC in their lifetime. Mostly, all of them show neoplasm and they are EACs. BDII rat strain is hormone dependent, so it could make an ideal model for hormonal carcinogenesis. There is a strong relation between marker alleles and tumor incidence in cross progenies. It includes susceptible BDII strain and non-susceptible strains, which was derived by using genome-wide screening with microsatellites. It was found that the tumors could be developed in inter strain crosses and it was not just because of susceptibility of alleles but also due to non-susceptible strains [3, 4].
Growth and receptors

The FGF family contains 18 secreted ligands and readily moves out to extracellular matrix by heparan sulfate proteoglycans (HPSG) with the help of proteases or particular specific binding protein. FGFs are released via the signal transmission so that it readily binds to the cell surface and forms a complex. The complex contains FGF, FGFR, and HPSG. Hormonal FGF have low attraction for heparin molecules and trust on klotho proteins as fundamental tissue-selective cofactors for binding to their similar FGFR. There are a total of 5 FGFRs, 1 to 4 of which are highly preserved for single-pass transmembrane tyrosine kinase receptors. There are many FGF signaling pathways for various functions such as cell proliferation, cell survival, and cell motility etc. [9].

a. Signaling pathways for cell proliferation

Dimerization of the ternary FGF, FGFR, and HPSG complex leads to conformational shift in the FGFR structure, resulting in intermolecular transphosphorylation of the intracellular...
tyrosine kinase domain and carboxy-terminal tail. Subsequent downstream signaling occurs through main pathways via interacellular receptor substrates FGFR substrate and phospholipase leading to upregulation of RAS dependent mitogen activated protein kinase (MAPK) and Ras independent phosphoinositid 3 kinase (PI3K) activated by FGFR [10]. FGF ligand binding to the FGFRceptor leads to activation of the FGFR kinase resulting in phosphorylation and activation of the FRS gene. The FRS gene in turn leads to activation of GRB2, SOS, and RAS consecutively. Activation of the Rasthrough the MAPK pathway acts on the target gene FOS which is responsible for including cell proliferation. The SPRY (sprouty proteins) released by the activation of the FGFR kinase act as negative feedback regulators by inhibiting the genes FRS and Ras. [10]

b. Signaling pathways for cell survival

![Signaling pathways resulting in cell survival.](image)

Activation of FGFRs also leads phosphorylation of the docking protein FGFR substrate 2(FRS2) followed by the recruitment of ship2 tyrosin phosphate, whereby the subsequent it association with growth receptor-bound2 (Grb2) and SOS. This complex triggers the
induction of the Ras/MEK/MAPK signaling pathway. The tyrosine phosphorylation of \( FRS2\alpha \) gene also mediates the recruitment of Grb2 and Grb1 resulting activation of PI3-kinase.

FGF ligand binding to the FGF receptor leads to activation of the FGFR kinase resulting in phosphorylation and activation of the \( FRS2\alpha \) gene. The \( FRS2\alpha \) gene in turn leads to activation of Grb2, Gab1, and PI3K consecutively. Activation of the PI3K through the PDK pathway (along with AKT) acts on the target gene \( FOXO \) which is responsible for cell survival. The \( CASPASES, BAX \) and \( BAD \) which are proapoptotic genes inhibit the gene \( AKT \) affecting cell survival. [10]

c. FGF signaling pathway

![Image of FGF Signaling Pathway](image)

Figure 3. FGF Signaling Pathway.

FGF induce FGFR mediated signaling pathways by interacting with specific FGFR and HSPG the micromolecularinteration mediate FGFR dimerization or oligomerization and activate multiple signal transduction pathways.

\( FGF \) gene binding to the FGF receptor,Leads to activation of the FGFR kinase resulting in phosphorylation and activation of the \( PLC\gamma \) gene. The \( PLC\gamma \) gene in turn causes dephosphorylation of DAG through conversion of PIP2 to PIP3. The desphosphorylated DAG results in activation of PKC and RAF consecutively. Activation of the RAF, through the
MEK kinase (along with ERK) pathway acts on the target gene. This is one of the other signaling pathways used by FGFR. The MPK3 acts as inhibitor to this pathway by inhibiting ERK gene. [10]

In dysregulation mechanism activation of ligand leads to over production of FGF genes and alters specificity of endogenous FGF genes and production of FGFR splices variants. Mutation in proteins involved in FGFR internalization can cause increased signaling. Ligands independent dysregulation mechanism leads to mutation, receptor dimerisation and translocation. Dysregulation of FGFR-dependent signaling can contribute to tumor growth and angiogenesis.

In embryogenesis one of the first areas in which FGF signalling was shown to be important in cell proliferation and migration was during wound healing. Initially it shows the key role for FGF signalling in epithelial is to repair but some FGF2 are important for the supply of new blood vessel growth at wound the site delivering oxygen nutrients in tumour angiogenesis. Some clinical diseases and involved mechanism of dysregulation of the FGFR receptor are shown in Table 1[10].

Table 1.

**Genetic alterations in FGF receptors and FGFs related to cancers**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Type of Dysregulation</th>
<th>Clinical Disease</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>Amplification</td>
<td>22% squamous small lung cancer. 10% breast cancer. Human melanoma</td>
<td>Over expression leading independent signaling.[11] FGF2-FGFR1 feedback loop.[12-13]</td>
</tr>
<tr>
<td></td>
<td>Autocrine signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>Activating mutation</td>
<td>12% endometrial cancer</td>
<td>activation mutation in extracellular α kinase domain.[14] deletion of coding axon located to proximal to c-terminal impeding receptor internalization.[15]</td>
</tr>
<tr>
<td></td>
<td>Gene amplification</td>
<td>Gastric cancer cell line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Altered spicing</td>
<td>Bladder prostate cancer</td>
<td></td>
</tr>
<tr>
<td>FGFR3</td>
<td>Activating mutation</td>
<td>Translocation</td>
<td>50-60% of non-muscle invasive and 17% of high grade bladder cancer. 10-20% multiple myeloma[18,19]</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------</td>
<td>--------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>FGFR4</td>
<td>Activating mutation</td>
<td>Childhood soft tissue sarcoma. Rhasdomepsarcoma.</td>
<td>kinase domain mutation causing autophosphorylation and constitutive signaling. [20]</td>
</tr>
</tbody>
</table>

**Autocrine signaling**
- FGF5
- FGF2, FGF8, FGF17, FGF18
- FGF19, FGF3,4, cyclin D

Tumor cell lines[21]  
Initiate Autocrine growth stimulation.[22,23]

**Paracrine Signalling**
- FGF2, FGF1, FGF2, FGF4, FGF5, FGF8, FGF18.

Small cell lung cancer.[25]  
Promote tumor neoangiogenesis.[26]

The aim of the present study was to investigate the gene expression of FGF in premalignant endometrial tissue compared to malignant endometrium in a BDII rat model of human endometrial cancer cell line.

The specific objectives of this thesis were:
• To investigate the gene expression of FGF in premalignant and malignant endometrial cancer cell lines in BDII rat model of human endometrial cancer cell lines.

• To compare the relative expression in premalignant and malignant endometrial cell lines with use of rat embryo fibroblast (REF).

2. Materials and methods

Animal crosses and experimental materials

BDII/Han inbred rat model was used for the study of human endometrium adenocarcinoma, along with the other two inbred rat strains SPRDCu3/Han and BN/Han. The non-susceptible BN or SPRD male’s crossed with BDII females toproduces F1 progenies. F1 was also backcrossed with BDII females to produces N1 progenies and by brother sister mating F2 progenies were produced In N1, F1 and F2 female progenies suspected tumors were removed surgically for the pathological study of its characterization. Tumor cell lines were established from the removed tumor however, some of the suspected tumor, could not be pathological diagnosed as endometrial cancer, and are probably pre-cancerous lesions, denoted as non-malignant tumors (NMT) in the present study[27].

In this work we also examined the FGF expression in the human EAC cell line Ishikawa (sigma Aldrich) and in an human embryonic kidney (HEK) cell lines (sigma Aldrich).

Human embryonic kidney cell line

The human embryonic kidney cells (HEK) were originally isolated from primary human embryo kidney cells. Human 293 cells were initiated to stain strongly and in particular with a monoclonal antibody to neurofilament[5]. The HEK cell line is used for expression equipment of recombinant proteins. Even if it had epithelial origin, the biochemical machinery is capable of carrying out different post translation folding to produce functional, mature protein with wide spectrum of non-mammalian and mammalian nucleic acids. This cell type was also seen to transform cells to study a mixture of cell-biological questions in neurobiology. The HEK cell is a popular choice among electro physiologists to study isolated receptor channels that include easy and fast reproduction and maintenance and also transfixion using a wide variety of methods like high efficiency of transfixion and protein production[6].
Ishikawa cell line

Various techniques are available to culture the human cell lines. Ishikawa cell line plays a vital role in the research of carcinoma with the help of various experimental techniques. In respect to endometrial carcinoma, a single human cancer cell might not contain all properties for the study. However, researchers were able to find out the well differentiated endometrial adenocarcinoma cell line named as ishikawa cell line (cancerous). This particular cell line had the special feature of containing both estrogen and progesterone receptors. When these cells are maintained for a longer period of time, they have tendency to transform into undifferentiated cells. This cell line is used in various research areas like molecular science, bio medicine and reproductive biology [7].

In this study, gene expression of fibroblast growth factor in endometrium cancer was studied. These cell lines are classified as malignant, non-malignant cell lines and in table 2, information (genetic background, pathology and tissue of origin) about the cell lines used is presented.

Table 2
EAC cells lines used in the gene expression profiling experiments

<table>
<thead>
<tr>
<th></th>
<th>Cells</th>
<th>Pathology</th>
<th>Genetic background</th>
<th>Tissue origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NUT 6</td>
<td>Endometrial carcinoma</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT Endometrium</td>
</tr>
<tr>
<td>2</td>
<td>NUT43</td>
<td>Endometrial carcinoma</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT Endometrium</td>
</tr>
<tr>
<td>3</td>
<td>NUT128</td>
<td>Endometrial carcinoma</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT Endometrium</td>
</tr>
<tr>
<td>4</td>
<td>NUT97</td>
<td>Endometrial carcinoma</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT Endometrium</td>
</tr>
<tr>
<td>5</td>
<td>Ishikawa</td>
<td>(HUMAN)</td>
<td></td>
<td>Endometrium</td>
</tr>
<tr>
<td>6</td>
<td>NUT75</td>
<td>Non-malignant tumors</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT Endometrium</td>
</tr>
<tr>
<td>7</td>
<td>NUT110</td>
<td>Non-malignant</td>
<td>(BDIIxBN)xBDII</td>
<td>RAT</td>
</tr>
</tbody>
</table>
In vitro cell culture conditions

Primary cell culture established from HEK, Ishikawa, EAC and NME tumors were propagated in RPMI 1640 media growth 500ml was prepared. In this experiment the freeze cell lines of rats as well as human was used and followed the cryogenic preservation of animal cells protocol.[28]

The cells were grown at 37°C in the atmosphere of 95% humidity and 5% CO₂ and harvested by trypsinization at confluence of 80-90%. The cells were then observed under the microscope. The detachment of cell by action of trypsin was observed. To stop the action of trypsin 3ml of media was added then the mixture was placed for centrifugation (4 min in 100xg) after centrifugation the supernatant solution was discarded and media was added into cell culture flask. It was resuspended for 4 to 5 times. The sample was placed in fresh cell culture tube and centrifuged, 40µl of sample mixed with tryphan blue which help in counting, 20µl were added into counting chamber hemacytometer. After counting remained cell culture was frozen[27, 28].

RNA extraction

Before the RNA extraction bench wiped with ethanol subsequently cleans with RNAase Zap (applied Biosystems) the protocol of applied Biosystems was followed. Cell pellet was washed with 1ml PBS, centrifuged for 5min at 10000 rpm also supernatant was discarded, the rest of cell pellet washed with lysis binding solution and vortex it. The 60µl RNA
homogenate additive leave mixture on ice for 10 min afterward 600µl of acid-phenol-chloroform was added. Then mixture was vortexed for 1min along with centrifuged for 5min at 10000rpm. Carefully aqueous phase was removed after that transferred in to fresh tube.

In this study, RNA extraction was performed by using mirVana™ PARIS™ RNA (total RNA) kit (Applied Biosystems California, United States) in RNAase free environment. The protocol was followed as mentioned in the manufacter’s protocol. The eluted solution from RNA isolation was collected and concentration and purity of every sample was measured through Nanodrop ND-1000 (Nanodrop Technologies, USA). The RNA was stored at -20°C until use.

**Reverse Transcription**

Reverse transcription high–capacity RNA-to-cDNA kit from Applied Biosystems (Life Technologies USA) was used for conversion of RNA to cDNA. The cells were thawed together with all the ingredients. 2µg of total RNA per 20µl reaction. The reverse transcription was performed as given in the manufacturer’s protocol.

The thermo cycle was programmed according to the instruction’s in the kit manual ( step 1: 60 minutes at 37°C, step 2: 5 minutes at 95°C and step 3: infinity at 4°C. After completion. The samples were stored in at -20°C.

**FGF gene expression assay**

In this study master mix containing buffer, enzyme, and nucleus free water (except sample) were transferred into a tube and centrifuged. UniversalTaqManmaster mixTaqMan probes (Applied Biosystems) were used. Subsequent to the addition of probes the master mix was centrifuged, cDNA samples were diluted in the ratio of 1:100 and A 96- well contain 11 µl of master mix and 9µl of sample were loaded in each well. Every sample was run in triplicates. Vortexed followed by centrifuged and then inserted in to thermocycler. The cycle for q-PCR were set at 50° for 2 minutes followed by 95° for 10 minutes, 95° for 15 seconds and 60° for 1 minute.

**Statistics**

Due to few numbers of samples, the statistics did not perform.
Result and discussion

Human pathological conditions are tightly associated with the deregulation of FGFR signaling. It includes various mechanisms like gene amplification, for example caused by single nucleotide polymorphism, chromosomal translocation and ligand availability. Deregulated gene expression is commonly observed in different type of human malignancies. This could be due to gene amplification or deregulation at transcriptional level [28, 29].

Many human diseases are driven in part by point mutations. The sequencing of cancer genomes has uncovered over many somatic mutation in coding exons of 518 human kinase genes. Most of the non-synonymous mutations involve FGF signalling and a few of these mutations was shown to exert a gain of function effect that contributes to the formation of abnormalities, such as uncontrolled growth and metastasis in various cancerous cells. The role of other mutations in tumorigenesis remains unknown [30]. Cancer cells may produce growth factors to which they produce responses, creating positive feedback signalling loop often termed autocrine stimulation [31]. The deregulation of negative regulators of FGFR signalling is commonly associated with the pathogenesis of various malignancies [32].

The aim of the present work is to investigate the expression of FGF genes by performing qPCR in premalignant and malignant endometrial cancer cell line in BDII rat model of human endometrial cancer cell line. The results are shown in Figure 5 and 6 as shown below.

![Graph showing FGF gene expression in malignant and non-malignant EAC cell lines samples.](image)

**Figure 5, Log2 FGFgene expression in malignant and non-malignant EAC cell lines samples.** The green bars, denote FGF expression in the malignant EAC samples, whereas the red bars denote FGF expression in non-malignant (NME) samples. In this study, the expression of gene FGF signaling was...
evaluated in malignant cell lines; NUT6, NUT43, NUT128, NUT97, and non-malignant cell line; NUT75, NUT110, NUT118, NUT129, respectively. No statistics has performed due to low number of samples.

By performing qPCR the expression of FGF has investigated at RNA level in malignant cell lines (NUT6, NUT43, NUT128 and NUT97) compare to non-malignant cell lines (NUT75, NUT110, NUT118 and NUT129). As shown in the figure 5, the expression of FGF in non-malignant cell lines denoted red bars, whereas malignant cell lines were denoted as green bars. In result, it has been found that the expression of FGF in non-malignant cell lines has a higher trend compare to malignant cell lines. Importantly, NUT 110, which is non-malignant cell line, has more trend of FGF and NUT6 which is a malignant cell line has very low trend of FGF compare to other cell lines. Overall, most of non-malignant cell lines has shown higher expression of FGF compare to malignant cell lines.

Over expression of FGF could be due to provoked ligand-independent signaling in endometrial carcinoma [33]. FGF gene amplification often leads to FGF over expression. The involvement of excessive FGF signaling in the initiation and progression of cancer and protective properties of FGF signaling against tumorigenesis have also been observed [33, 34]. However, it will be expedient to explore and identify the underlying mechanisms in differential effects which can lead to how FGF signaling can be most therapeutically targeted.

We found a FGF gene that was differentially expressed between two groups and different cell lines. In Figure 5, the differential expression was between EAC and NME cell lines. FGF gene expressed in cell line has a higher trend in the NME group higher trend could be amplification of FGF gene or by other forms of genetic alteration such as base deletion [34]. Point mutations were also observed in endometrial carcinoma [34]. The FGF gene was more or less normal in the EAC. It can be conspicuously seen that the FGF gene is higher trend in the non-malignant cell lines (red bars) when compared to the expression in the malignant cell lines (green bars). As here used very few samples cell lines can’t conclude about significant expression but higher FGF trend in NME suggest tumorigenesis [35]. Need additional technique with more number of samples to assess FGF gene.
Figure 6, FGF gene expression in HEK and Ishikawa cell line samples.

The light blue bars, denote FGF gene expression in the HEK cell lines, whereas the dark blue bars denote FGF expression in Ishikawa cell lines (figure 6). It can be observed that there is expression of gene FGF signaling in HEK cell line is lower as compare with Ishikawa cell line has higher trend (figure 6).

Most of studies relate activation of FGF signaling with oncogenesis but there is undeniable evidence from mouse model for tumor suppressive role of FGF. In mouse models of medulloblastoma, FGF signaling inhibits sonic hedgehog signaling and this blocks the proliferation of cancer cells. In several studies, human tumors and cancer cell lines efficiently came from tissue where kidneys support the tumors protective effect of FGF signaling. The FGF expression in bladder cell lines block the proliferation physiologically expressed in epithelial structures and they are downregulated on progression in prostate cancer, bladder cancer and salivary adenocarcinomas [35].

In result, it has been found that the expression of FGF in Ishikawa cell lines has a higher trend compare to HEK cell lines. In figure 6, it can be conspicuously seen that the FGF gene is highly expressed in the Ishikawa cell line as compare with HEK. The well differentiated endometrial adenocarcinoma cell line named as Ishikawa were FGF has shown higher
trend. It containing both estrogen and progesterone receptors cell line. The uncontrolled growth of this subpopulation was proposed to be driven by estrogen hormone stimulation, which in turn, regulates theparacrine signaling of FGF9–FGFR3 [7] but need additional technique to assess FGF gene. Over expression and different dysregulation like amplification, Autocrine signaling of the gene FGFR leads to various disease such as lung cancer, breast cancer and human melanoma. After amplification, FGFR was overly expressed producing independent signaling as shown (table 7).

**Table 7 Involvement of FGF dysregulation in clinical diseases**

<table>
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<td>Gastric cancer cell line</td>
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</table>

**General discussion**

The important role of FGFR signaling in tumorigenesis, various approaches developed for targeting the upstream and downstream axis of the signaling and many novel therapeutic molecules are introduced and currently trials are going on in preclinical and clinical trials with different FGFR associated tumor. Molecules are targeted at FGF ligands or receptors [35]. Targeting the FGFR family, they inhibit vascular endothelial growth factor receptors and also platelet-derived growth factor receptors [36, 37]. Some of the compounds such as TKI258 and AZD4547 were demonstrated as positive potential inhibitors of FGFRs and this was tested in advanced clinical trial. Dovitinib showed high potency against most FGFRs [38, 39].
Dovitinib molecule is currently tested in phase 3 clinical trial for renal cell carcinoma and phase 2 clinical trial for endometrial cancer, breast cancer and also urothelial cancer[39]. So dovitinib could possibly exert anticancer effects. By targeting FGFRs or regulators of angiogenesis, endometrial cancer patients with or without FGFR2 mutation are tested separately to show its mechanism of function[40].

**Conclusions and future perspective**

The conclusion was that there is higher trend of the FGF gene in the non-malignant cell lines, and difference in the expression of Ishikawa cell line when compared to HEK cell line. Its suggested that the over expression of FGF gene has a role in tumorigenesis in endometrial carcinoma but cannot conclude about significantly over expression of FGF gene in this study. Reasons very few number of samples, not performed any statistics need additional technique to assess FGF gene. However it would be of utmost importance to consider the possible effect of an antagonist of FGF ligand, that has high efficiency to the FGFR but elicit low efficacy. This could in a way, check the effect of over expression of FGF in tumorigenesis. There is a need of further studies to validate the results of FGF gene in the pathogenesis of the endometrial carcinoma with more number of samples and also with the different histological types of endometrial cancer and additional techniques for Further experiments have to be designed and carried out to develop targeted therapies.

**Acknowledgements**

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Furthermore, I would like to thank Dr. Sandra Karlsson, Head of the Bio-medicine department for all the words of encouragement and useful comments through this master thesis.

Last but not least, I would like to express gratitude to all my friends and family who have helped me and extended their support throughout.
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35. Zisman-Rozen, S. F. D.-I. (2007). Downregulation of Sef in a variety of human carcinomas. Inhibitor of receptor tyrosine kinase signaling, is common to a. In Oncogene (pp. 6093–6098.).


**APPENDIX**

**Appendix 1**

Before entering in to aseptic area shoes protector was wired, clean hand with 70% ethanol wired the proper gloves and open the laminar fan was stared clean it with the solution, pipette, marker, PCR tubes was collected inside the laminar uvlight was stared do not put the protein media inside the ultra violet light media was kept in 4°C temp. RPMI 1640 media growth 500ml was prepared containing ingredient, HEK freezing cells from rats was provided by lab supervisor. ml of PBS was added to HEK cells then placed for centrifugation for 4 min in 100xG the supernatant solution was discarded and placed the cell for resuspension inside the 3ml of media growth, after that take a 10ml cultural flask 7ml of media growth was added and again resuspended in 3ml of media growth then cells was observed under the microscope for clumps and then it was placed inside the incubator in 37°C and 5% carbon dioxide

**Appendix 2**

**Table 1. Ingredient used for the preparation of culture media.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Stock solution</th>
<th>Working solution</th>
<th>ml of stock solution working</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture media ppmi 1640 (500ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPMI(Roswell.park Memorial institute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-glutamine (not stable)</td>
<td>200mm</td>
<td>2mm</td>
<td>5ml</td>
</tr>
<tr>
<td>Hepes</td>
<td>1mm</td>
<td>10mm</td>
<td>5ml</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>100ml</td>
<td>1mm</td>
<td>5ml</td>
</tr>
<tr>
<td>Pest (penicilliumsloruptomiaire)</td>
<td>100x</td>
<td>1x</td>
<td>5ml</td>
</tr>
</tbody>
</table>
Reverse Transcription:

For the reverse transcription high –capacity RNA-to-cDNA kit from Applied Biosystems was used were thawed all the ingredients showed in table below 20µl sample was prepared with the help of kits intrusions all the components were placed in to PCR tube and spin dowtube and kept in to ice. For negative control HEK cells was used.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>+RT</th>
<th>-RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X RT buffer</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>20x RT Enzyme mix</td>
<td>1.0</td>
<td>-HEK</td>
</tr>
<tr>
<td>Nucleus free water</td>
<td>8.5</td>
<td>to 20µl</td>
</tr>
<tr>
<td>Sample</td>
<td>0.5</td>
<td>9µl</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

The thermo cycle was programmed following kit intrusions. As showed in table below thermo cycle was ran by keeping in to following temperatures and after completion of thermo cycle sample was kept in to -20°C.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step2</th>
<th>Step3</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>95°C</td>
<td>4°C</td>
</tr>
<tr>
<td>60 min</td>
<td>5min</td>
<td>Infinity 20 min</td>
</tr>
</tbody>
</table>

Appendix 3

Q-PCR:

Prepared Master Mix was transferred in to tube and centrifuged. TaqMan probes was added in to the master mix and kept for centrifugation. cDNA samples was dilulated in to 1:100 and 96- well contain 11 µl of master mix, and 9µl of sample were loaded in each well and every
sampled was loaded in triplet, kept for vortexed and centrifuged and then inserted in to thermo cycler it was already programmed and reaction was carried out.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>2min</td>
<td>10min</td>
<td>15sec</td>
<td>1min</td>
</tr>
<tr>
<td>50°C</td>
<td>95°C</td>
<td>95°C</td>
<td>60°C</td>
</tr>
</tbody>
</table>

Appendix 4

**Below table concentration, ratio A260/280 and A230/230Vmeasured by Nanodrop**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Concentration (mg/ml)</th>
<th>A260/280</th>
<th>A260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishikawa</td>
<td>83.0</td>
<td>2.17</td>
<td>1.25</td>
</tr>
<tr>
<td>HEK</td>
<td>78.6</td>
<td>2.10</td>
<td>1.92</td>
</tr>
<tr>
<td>NUT 6</td>
<td>102.2</td>
<td>2.18</td>
<td>1.98</td>
</tr>
<tr>
<td>NUT 43</td>
<td>250.3</td>
<td>2.10</td>
<td>1.69</td>
</tr>
<tr>
<td>NUT 128</td>
<td>85.1</td>
<td>2.12</td>
<td>2.16</td>
</tr>
<tr>
<td>NUT 97</td>
<td>123.5</td>
<td>2.18</td>
<td>1.54</td>
</tr>
<tr>
<td>NUT 75</td>
<td>136.8</td>
<td>2.17</td>
<td>1.79</td>
</tr>
<tr>
<td>NUT 110</td>
<td>76.8</td>
<td>2.06</td>
<td>1.89</td>
</tr>
<tr>
<td>NUT 118</td>
<td>198.7</td>
<td>2.19</td>
<td>1.80</td>
</tr>
<tr>
<td>NUT 129</td>
<td>143.9</td>
<td>2.07</td>
<td>1.98</td>
</tr>
</tbody>
</table>