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REVIEW

Focal Adhesion Kinase Related Gynecologic Cancer: a review

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ABSTRACT

Gynecologic cancer is a major health burden in women across the world. Almost a half million mortalities from gynecologic cancer were reported every year. Knowledge in molecular biology research on cancer could lead to develope novel therapeutic options in gynecologic cancer. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase which plays an essential role in cancer cells survival, migration, invasion, metastasis and also tumor angiogenesis. Various human cancerous tissues were demonstrated over expression of FAK which were correlated to the survival. There were evidences showed that FAK involved in molecular pathways of ovarian cancer, endometrial cancer and cervical cancer. In the light of this review, FAK has been a promising mediator that regulates cancer growth and metastasis. Therefore, molecular therapies targeted FAK may be beneficial for patients with gynecologic cancer in the near future.

Key words: focal adhesion kinase, gynecologic cancer

Introduction

Gynecologic cancer is a major health burden in women around the world. More than million women suffered from gynecologic cancer in the year 2008[1]. Unfortunately, the incidences trend to increase from the report in 2002[2]. Almost a half million mortalities from gynecologic cancer were also reported[1]. Most of patients with gynecologic cancer were diagnosed at advanced diseases. Some patients resisted to primary therapies. Almost all of the patients with recurrent diseases resisted to salvaged therapies, so they have never cured. To date, there have been many efforts to understand biology and etiology of cancer for developing novel more effective treatment options.

Directly molecular targeting to cancer cells plays an important role for developing modern effective therapies with less toxicities to the host cells. Tyrosine kinase is one of the attractive targets which regulate cell differentiation, growth and metabolism in various molecular pathways. Focal adhesion kinase (FAK) was identified in Src-transformed fibroblasts in 1992[3,4]. A 125-kDa FAK's structure composes of the NH2 terminus by the four-point-one, ezrin, radixin, moesin (FERM) domain, kinase domain, COOH-terminal proline-rich and focal adhesion targeting (FAT) domain[5-7]. The FERM domain serves as a main interacting function. FAK is a non-receptor intracytoplasmic protein. A variety of mechanisms can activate FAK including integrin receptors, G protein-coupled receptors and Src. Integrins are transmembrane receptors which contact

to extracellular matrix and transmit growth factors signals and FAK is a major regulator of integrin signal in cancer cells[8]. Autophosphorylation on tyrosine Y397FAK following the integrin signal is an important cancer regulation in combination with the 85 kDa subunit of phosphatidylinositol 3-kinase (PI3K) in PI3K/Akt pathway[9]. Over expression of FAK was shown in many human cancers including breast, colorectal, sarcoma, head and neck (HNSCC), prostate, and thyroid which correlated with survival outcomes[10].

Scientists studied roles of FAK in cells survival[11], migration[12], invasion[13] and also tumor angiogenesis[14]. Almost twenty years of FAK focused publications in literatures, FAK related cancer was discovered and FAK inhibitor has been developed. It is attractive to review FAK related gynecologic cancer in an aspect of molecular functions and applications.

Ovarian cancer and FAK

Since 1999, studies using immunohistochemistry of human ovarian cancer tissues demonstrated over expression of FAK[15-18]. A fourfold increasing FAK levels in most invasive ovarian adenocarcinomas compared with normal ovarian tissues was discovered[15]. FAK was revealed its important role of migration and invasion in ovarian cancer. Cancer cells migration, a repeating process of adhesion and detachment with the extracellular microenvironment, is a priming process of tumor metastasis. The patients with high tumor stage, high tumor grade, positive lymph nodes and presence of distance metastasis were significantly associated with overexpression of FAK[18]. The shorter overall survivals were also correlated. Furthermore, at cellular level, inhibiting FAK phosphorylation resulted in decreased migration and invasion[18].

FAK silencing study was shown in vitro inhibition ovarian cancer cells survival in both taxane-sensitive and taxane-resistant cells. Chemotherapy-induced apoptosis followed activated caspases in various cancer cells, and Docetaxel induced FAK cleavage though activation of caspase-3 in taxane-sensitive ovarian cancer cells was demonstrated[19]. Another study[20] combined Docetaxel and FAK gene silencing, with short interfering RNA (siRNA) technology using a neutral lipid nanoliposome, dioleoyl phosphatidylcoline (DOPC). It was resulted in decrease microvessel density, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 expression, and increase apoptosis of ovarian tumor in animal model. Therapeutic FAK inhibitor was effective alone or combination with chemotherapy in both taxane-sensitive and taxane-resistant cells in vitro cytotoxicity experiments, Western blot analysis and also in vivo xenograft mice models[21].

FAK regulates various cancer molecular cascades. In ovarian cancer, FAK was shown coordinated function with ephrin receptor A2(EphA2)[22]. Combined knock-down of FAK gene and EphA2 gene using liposomal siRNA resulted in reducing angiogenesis and tumor cell proliferation in an orthotopic ovarian cancer model. FAK also interacted with the transcriptional coactivator the Four and a Half LIM domain protein 2 (FHL2) which is reacted with integrin in immunoprecipitation study and both (FAK and EphA2) were overexpressed in epithelial ovarian cancer in immunohistochemistry study in 2004[16].

Expression of phosphorylated Y397FAK was studied with human protease activated receptors 1 (hPar1), a family of G-protein coupled receptors (GPCR)[17]. They were demonstrated the differential expression pattern of hPar1 in human invasive, low malignant potential ovarian tissues and high levels of phosphorylated Y397FAK in invasive ovarian cancer but not in normal ovarian tissue. They might cooperate to promote ovarian cancer.

The study in 2008[23] was shown that apigenin, a flavonoid which is widely distributed in many fruits and vegetables, impaired FAK expression and also inhibited in vitro migration and invasion of ovarian cancer cells.

Chronic stress is associated with hormonal changes either norepinephrine or epinephrine, they both presented in the ovary. Normal cells having a process entering apoptosis after detached and separated from extracellular matrix and neighboring cells are called anoikis. The restraint stress in an orthotopic human ovarian cancer mouse model was studied. It was demonstrated that increasing in

norepinephrine and epinephrine protected the tumor cells from anoikis and promoted their growth by activating phosphorylation of Y397FAK[24].

Recent study in 2010[25], using ovarian cancer ascites induced phosphorylation of FAK, which closely correlated with the phosphorylation of Akt in phosphatidylinositol 3-kinase/ a serine/threonine protein kinase (PI3K/Akt) pathway for cell survival. Inhibition of FAK phosphorylation by alpha-v-beta 5 ($\alpha v\beta 5$) the integrin-blocking antibodies and knock-down FAK by RNA interference were also associated with inhibition of ascites-mediated Akt activation and cells survival[25]. This study showed the important roles of FAK in ovarian cancer.

Endometrial cancer and FAK

Indistriction from ovarian cancer and other cancers, endometrial neoplasia was shown overexpression of FAK. Tissue microarray of human endometrial carcinoma including endometrioid, serous and clear cell carcinoma were immunostained and studied. Upregulation of FAK was demonstrated. Highest rate was seen in type II endometrial carcinoma, serous and clear cell carcinoma[26]. Another study showed increased expression of FAK correlated with higher histological tumor grade[27].

FAK and Gonadotropin-releasing hormone (GnRH) were studied in vitro in 2009[28]. GnRH-I and GnRH-II may be involved in the inhibition of endometrial cancer cell growth via activation of integrin beta3 and FAK.

The role of FAK in endometrial cancer is not fully understood because of limited studies. Most endometrial cancer are type I which have mutation of phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a tumor suppressor gene. PTEN plays the main role in dephosphorylated phosphatidylinositol 3, 4, 5-triphosphate (PIP3) which down regulation PI3K/Akt pathway for cancer survival. PTEN also dephosphorylated FAK. This is an interesting function to be study for FAK related endometrial cancer.

Cervical cancer and FAK

Cervical cancer is the most common cancer in women in developing world. Unfortunately, more than half of them died within a year because of advanced and metastatic disease. Thus, it is needed to control. Understanding molecular mediators is an anchorage for controlling cancer cells survive and metastasis. FAK is an interesting one of the targets.

Since 1997, it was shown that FAK expression was significantly elevated in human papilloma virus (HPV)-18 infected human genital epithelial cells[29]. The study in 2003[30] demonstrated an elevated tyrosine phosphorylation of FAK in cervical carcinoma and related to the cancer invasion. Another study in 2003[31] also reported that most of the invasive squamous cell carcinomas (SCCs) of the cervix (13 of 16 cases) were positive for FAK. Minimal FAK expression in benign diseases and low-grade squamous carcinoma intraepithelial lesion (CIN I-II), and variable FAK expression in carcinoma in situ (CIS) lesions were found. Using immunohistochemistry, phosphotyrosine-containing protein localized to FAK (pp125FAK) expression in cervical cancer was studied[32]. In contrast, the cervical cancer with weak expression of FAK was a strong independent predictor of poor therapeutic outcome.

As described, phosphorylation Y397 site of the FAK is an important site which is interested to study this expression in cervical cancer in further studies.

Other gynecologic cancer

Various types of sarcoma including fibrosarcoma, rhabdomyosarcoma and osteosarcoma were shown expression and activities of FAK[33]. Uterine leiomyosarcoma may be shown some expression of FAK and some responses to FAK inhibitor in future trials. Therefore, molecular signal transduction system of FAK involved carcinogenesis, survival and metastasis of the sarcoma cells are needed to understand.

Priming studies of FAK were using trophoblastic cells. Human trophoblastic cells adhesion and spreading were inhibited by [4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3, 4-d]pyrimidine] (PP2), a Src family kinase (SFK) specific inhibitor in the study in 2008[34]. FAK phosphorylation inhibition at many sites including tyrosine Y397 site was demonstrated. Another study

showed that the expression of FAK in trophoblastic cells promoted cells invasion, which is the behavior of cancer[35]. Thus, gestational trophoblastic tumor have been interesting which could be related to FAK. As described, many epithelial cancers related to FAK. Vulva and vaginal cancer which are solid epithelial tumor have never been studied. So these topics will be interesting in the future. The summary of FAK related articles in gynecologic cancer was shown in Table 1.

Table 1. FAK related articles in gynecologic cancer

Year	Authors	Descriptions of articles	Type of cancer
1997	McCormack SJ, et al[29].	Activation of the FAK signal transduction pathway immortalized with human papillomavirus type 18.	Cervical
1999	Judson PL, et al[15].	Overexpression of FAK.	Ovarian
2003	Moon HS, et al[30].	The expression and tyrosine phosphorylation of E-cadherin/catenin adhesion complex, and FAK.	Cervical
	MH Oktay, et al[31].	Focal Adhesion Kinase as a Marker of Malignant Phenotype.	Cervical
2004	Livasy C. et al[26].	FAK overexpression.	Endometrial
	Gabriel B, et al[16].	FAK interacts with the transcriptional coactivator FHL2 and overexpressed.	Ovarian
	Sood AK, et al[18].	Biological significance of FAK: role in migration and invasion.	Ovarian
2005	Halder J, et al[19].	FAK silencing augments docetaxel-mediated apoptosis.	Ovarian
	Grisaru-Granovsky S, et al[17].	Differential expression of protease activated receptor 1 (Par1) and pY397FAK.	Ovarian
2006	Halder J, et al[20].	FAK targeting using in vivo short interfering RNA delivery in neutral liposomes.	Ovarian
	Gabriel B, et al[32].	Weak expression of FAK (pp125FAK) associated with poor disease outcome.	Cervical
2007	Halder J, et al[21].	Therapeutic efficacy of a novel FAK inhibitor TAE226.	Ovarian
2008	Hu X, et al[23].	Apigenin inhibited migration and invasion of human ovarian cancer A2780 cells through FAK.	Ovarian
2009	Gabriel B, et al[27].	Expression of FAK.	Endometrial
	Park DW, et al[28].	Gonadotropin-releasing hormone (GnRH)-I and GnRH-II induce cell growth inhibition: involvement of integrin beta3 and FAK.	Endometrial
	Shahzad MM, et al[22].	Dual targeting of EphA2 and FAK.	Ovarian
2010	Sood AK, et al[24].	Adrenergic modulation of FAK protects human ovarian cancer cells from anoikis.	Ovarian
	Lane D, et al[25].	Ascites protects from TRAIL-induced cell death through $\alpha v\beta 5$ integrin-mediated FAK and Akt activation.	Ovarian

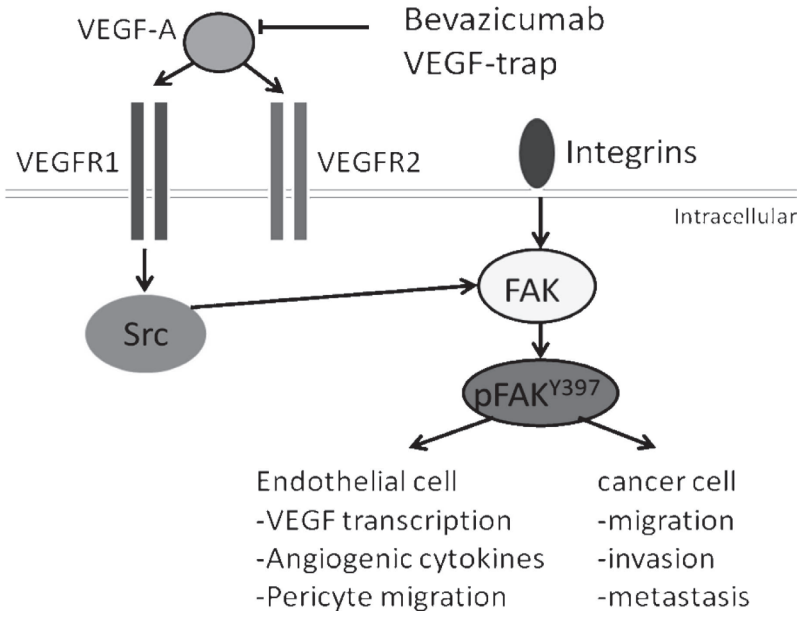

Fig. 1. FAK molecular pathway in cancer cell and tumor associate-endothelial cell. Bevazicumab and VEGF-trap act on VEGF-A ligand inhibition. Intracellular non-receptor FAK plays roles on cancer cell and tumor associate-endothelial cell for cancer cell migration, invasion, metastasis and angiogenesis.
(FAK = Focal adhesion kinase, VEGF = vascular endothelial growth factor)

Challenges and directions

This review showed FAK over expression in gynecologic cancer which related to poor survival. Many FAK inhibitors has been developed and studied both in vitro and in vivo animal models. FAK inhibitors alone or in combination with conventional chemotherapeutic agents have shown a number of response in preclinical cells and in vivo animal studies[6,21,23]. It might be intriguing to combine with small molecule tyrosine kinase inhibitors or anti-angiogenic therapies to increase cancer cells responses. Development of FAK inhibitors in clinical trials will become emerging a provital target for successful gynecologic cancer control. The efficacy of FAK inhibitor for cancer control has to be proven. It will be open up the therapeutic options for patients with gynecologic cancer in the near future.

Conclusion

Since almost two decades, FAK has been involved in molecular pathways of cancer. A number of studies have shown the relation of FAK in ovarian cancer, endometrial cancer and cervical cancer. Many molecular mechanisms of FAK in gynecologic cancer are still not fully understood. Continuing research studies to explore the role of FAK in gynecologic cancer are needed. In the light of this review, FAK has been a promising cancer regulation. Therefore, molecular therapies targeted FAK may be beneficial for patients with gynecologic cancer.

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Focal adhesion kinase ***สัมพันธ์กับมะเร็งนรีเวช***

ดวงมณี ธนัพประภัศร์

มะเร็งนรีเวชเป็นปัญหาสาธารณสุขที่สำคัญของสตรีทั่วโลก ในแต่ละปีมีสตรีได้รับการวินิจฉัยเป็นมะเร็งนรีเวชประมาณห้าแสนราย ความรู้จากการศึกษาวิจัยทางชีวโมเลกุลของมะเร็งนำไปสู่การคิดค้นพัฒนายาและแนวทางการรักษามะเร็งนรีเวชที่มีประสิทธิภาพแบบใหม่ Focal adhesion kinase (FAK) เป็นโปรตีน non-receptor tyrosine kinase ชนิดหนึ่ง ที่มีบทบาทสำคัญต่อการมีชีวิตรอดของเซลล์มะเร็ง การรุกแพร่กระจายของเซลล์มะเร็ง และการสร้างเส้นเลือดหล่อเลี้ยงเซลล์มะเร็ง มะเร็งหลายชนิดในมนุษย์พบมีโปรตีนนี้สูงมากและมีความสัมพันธ์กับการรอดชีพของผู้ป่วยที่ลดลง มีหลักฐานแสดงให้เห็นว่าโปรตีนนี้มีส่วนเกี่ยวข้องกับมะเร็งรังไข่ มะเร็งเยื่อบุโพรงมดลูกและมะเร็งปากมดลูก จากการทบทวนการศึกษาต่างๆ นี้พบว่า FAK เป็นโปรตีนที่มีส่วนสำคัญในการควบคุมการเจริญเติบโต และการรุกแพร่กระจายของเซลล์มะเร็งในมะเร็งนรีเวช การรักษาด้วยยาต้าน Focal adhesion kinase อาจจะมีประโยชน์ต่อผู้ป่วยมะเร็งนรีเวชในอนาคตอันใกล้นี้