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IGFBP-3 與卵巢癌侵入機制之研究

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計畫主持人：童寶玲

計畫參與人員：林欽塘，黃奇英， Pinchas Cohen

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行政院國家科學委員會補助專題研究計畫成果報告

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中 華 民 國 94 年 10 月 4 日

Abstract:

To delineate the molecular signature in ovarian cancer invasion, we established and characterized a human ovarian endometrioid carcinoma (EC) cell line OVTW59-P0 and its invasion-related sublines (P1 to P4, in the order of increasing invasive activity). Genes correlated positively or negatively with invasion were identified from microarray analysis. Among these genes, IGFBP-3 exhibited a most significant migration-invasion suppressor signature. Ectopic expression of IGFBP-3 effectively inhibited cancer cells migration, invasion and metastasis through down regulation of the kinase activities of extracellular signal-regulated kinase (ERK). In 35 patients with ovarian EC, low IGFBP-3 expression correlated significantly with higher tumor grade, advanced stage and poor survival. This suggests that IGFBP-3 plays an important role in the regulation of cancer invasion and acts as a prognostic marker for ovarian EC.

Keywords: Ovarian endometrioid carcinoma, invasion, migration, metastasis, transfection, signal transduction.

Aim: To study the mechanism of migration/invasion inhibition in insulin-like growth factor binding protein-3 in ovarian endometrioid carcinoma.

Introduction:

Ovarian carcinoma is one of the leading causes of death in Taiwan and the number of cases has been increasing. Metastasis has occurred in the majority of patients by the time of diagnosis and prognosis is poor despite of recent advancement in study of tumor oncology. For identification of invasion related suppressor genes from ovarian carcinoma, we have successfully established an ovarian endometrioid carcinoma (EC) cell line OVTW59 and further established an ovarian cancer invasion model using transwell invasion chamber. P0 to P4 represented a series of ovarian cancer sublines with increasing invasion capabilities. Subsequently genes that correlated positively or negatively with ovarian cancer invasion were identified by cDNA microarray. These invasion-related genes were validated by real-time PCR, and IGFBP-3, one of the tumor suppressor genes, was selected in this study.

Insulin-like growth factor binding protein (IGFBP), a circulating transport protein for insulin-like growth factor (IGF)-I and IGF-II, are recognized in playing a variety of roles in the circulation, the extracellular environment, and inside the cells (reviewed in ref 1). IGF are essential for normal embryonic and postnatal growth (2). Increased expressions of IGF-I, IGF-II, and IGF receptor (IGF-IR) or a combination thereof have been documented in many human malignancies (reviewed in ref 3). Moreover, IGF overexpression in cancer is associated with poor patient prognosis (4,5). The bioactivity of IGF is regulated by six known high affinity IGFBP (6), which bind the IGFs with equal or higher affinity than IGF-IR (7). The molar excess of IGFBPs, along with their higher affinity, leads to effective sequestration of IGFs by IGFBPs, resulting in little or no free IGFs in most biological systems. Therefore, the mitogenic activity of IGFs may be regulated at the level of IGFBP. The major IGF transport function can be attributed to IGFBP-3. From epidemiological studies, high levels of circulating IGF-I and low levels of IGFBP-3 are associated with increased risk of cancers, including those of the prostate, breast, colorectum, and lung (reviewed in ref 8).

Here, through in vitro and in vivo invasion parameters, we demonstrated a novel role of *IGFBP-3* as an invasion–migration suppressor in ovarian EC. In addition, we investigated the invasion-related signaling pathways of IGFBP-3. We showed that IGFBP-3 expression parallel with the down regulation of the kinase activities of extracellular signal-regulated kinase (ERK). The important role of IGFBP-3 as an invasion suppressor in ovarian EC cell is underlined by the fact that IGFBP-3

expression is suppressed in high-grade carcinoma and correlated significantly with survival in patients with ovarian EC.

Material and methods:

Chemical reagents: Cell culture reagents were purchased from Gibco/BRL (Grand Island, NY) and general laboratory reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Monoclonal antibody (mAb) directed against IGFBP-3 was purchased from Sigma-Aldrich (St. Louis, MO). The mAb against ERK1/2, Akt/PKB, phosphorylated ERK1/2 (Tyr^{204}) and phosphorylated Akt/PKB (Thr^{308}) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and mAb against tubulin was purchased from Neomarkers (Fremont, CA). PD98059 was purchased from EMD Biosciences (San Diego, CA). The lipofectamin 2000 was purchased from Invitrogen (Carlsbad, CA), and G418 from Calbiochem (Darmstadt, Germany). Normal horse serum and avidin-biotin-peroxidase complex were purchased from Vector Laboratories Inc (Peterboroug, UK).

In vitro invasion assay: The membrane invasion culture system (MICS) (9) was used with some modification. A polycarbonate membrane, containing 8 or 10 μm pores, (Nucleopore Corp., Pleasanton, CA) was coated with a mixture of 50 ug/ml laminin, 50 μg/ml type IV collagen and 2 mg/ml gelatin in 10 mM glacial acetic acid solution. The membrane was placed between the upper- and lower-well plates of the MICS chamber. Cells were resuspended in DMEM containing 10% FCS and seeded into the upper well of the chamber ($5x10^4$ cells per well). After incubation for 24 or 36 hours at 37ºC, cells that had invaded the coated membrane through the membrane pores were removed from the lower wells with 1 mM EDTA in PBS, and dot-blotted on a 3 μm polycarbonate membrane. Blotted cells were fixation in methanol, stained with Liu's staining solution and number of cells in each blot was microscopically counted.

Tumorigenicity: Severe combined immunodeficiency (SCID) mice, age 6 – 8 weeks, (5 for each group) were transplanted subcutaneously in the back with 2 to 3 X 10^7 cells. Size of tumor growth was measured using vernier calipers. Tumor volume was calculated by the formula, length X $width^2$ X 0.52, which approximates the volume of an elliptical solid mass. The tumors were removed after eight weeks, and the mice were autopsied for examination of visceral metastasis. Some tumor fragments were prepared for paraffin blocks, others were cultured in tissue culture system and subjected to chromosome analysis.

Western blotting: Either condition media (concentrated to 20 fold) or cell lysates

were used. Cells were extracted in lysis buffer (20 mM HEPES at pH 7.9, 0.4 N NaCl, 1 mM EGTA, 1 mM DTT, 0.5 %Triton-X100) with protease inhibitor [1 mM PMSF, and protease complete inhibitor cocktail (Roche, Mannheim, Germany)] on ice. Extracts were cleared by centrifugation and proteins were further extracted in lysis buffer with a lower concentration of salt (10 mM HEPES at pH 7.9, 10 mM of filtered KCl, 0.1 mM EGTA) with protease inhibitor. Twenty μg of protein from supernatants (concentration determined by Bio-Rad protein assay), or the concentrated culture media, were electrophoretically separated on a 8% SDS-PAGE and transferred to membranes (Schleicher & Schuell, Germany) using the MilliBlot-SDS semi-dry electroblotting system (Millipore, Bedford, MA). After blocking of non-specific binding sites using a blocking solution [5% nonfat milk, 0.9% NaCl, 0.1% Tween-20, 10 mM Tris-HCL (pH 7.4), 0.002% antifoam A], membranes were incubated with primary antibodies in PBS with 0.05% Tween-20 and blocking reagent at 4°C overnight. Reactions were amplified by incubation with biotinylated second antibody followed by streptavidin-HRP. Results were exposed to a film using ECL system.

Transfections: IGFBP-3 transfections were carried out using lipofectamin 2000. The constitutive expression vector, pKIGFBP-3, containing the full-length coding sequence of the hIGFBP-3 cDNA under the control of the hβ actin promoter, and the SV2-neo selection gene (containing resistance to G418) was used as previously described (10). The pK plasmid without hIGFBP-3 insert was used as control. 4 x 10^5 cells in 2 ml culture medium were seeded into six-well tissue culture plates 1 day before transfection, and then incubated with 6 μl of lipofectamin 2000 in 1 ml serum free medium containing 2 μg of plasmid. Cells were incubated for 6 hours at 37ºC in 10% CO_2. Stable lines expressing hIGFBP-3 or pK plasmid without hIGFBP-3 insert were then selected and established in DMEM solution with 10% FCS and 300 ug/ml G418. Transient transfection using IGFBP-3 RNA interference (short hairpin, shRNA) (Open Biosystems, Huntsville) was performed with the sequence of TGCTGTTGACAGTGAGCGCGCGCACAGGCTTTATCGAGAATAGTGAAGCC ACAGATGTATTCTCGATAAAGCCTGTGCGCATGCCTACTGCCTCGGA, according to the manufacturer's instruction. The control RNA interference was performed using scrambled oligo sequence.

Scratch assay: Confluent monolayers of cells or transfectants were maintained in culture medium for 24 hours. 200-μl plastic pipette tips were used to scratch the monolayers. After 17 hours, transfectants were photographed under an inverted microscope.

Immunohistochemical staining: Thirty-five cases of ovarian EC paraffin blocks were retrieved from the Department of Pathology, National Taiwan University Hospital from year 1994 to 2000 for immunohistochemical studies. Cases were treated and followed as in our previous report (11). Paraffin sections from xenograft tumors were also used for immunohistochemical studies. Four-μm paraffin sections were dewaxed, rehydrated, and pretreated with high temperature (pressure cooker) for antigen retrieval using 10mM citrate buffer (pH 6.0). The endogenous peroxidase was blocked by 1% H_2O_2 and the non-specific binding was blocked by normal horse serum. Primary antibodies were incubated overnight, followed by biotinylated secondary antibodies. Then the avidin-biotin-peroxidase complex was applied and the substrate reaction for peroxidase was developed with 0.05% 3,3'-diamino-benzidine (DAB) tetrahydrochloride solution containing 0.01% H_2O_2 at room temperature. Slides were dehydrated and mounted in Permount solution.

Statistical analysis

Chi-square test was used to correlate the IGFBP-3 expression patterns and tumor recurrence with age and the clinicopathological features. The associations of IGFBP-3 expressions with patient overall and disease-free survival rates were assessed by Kaplan-Meier analysis and the differences were tested for significance by the log-rank test. Multivariate regression analysis was used to evaluate IGFBP-3 expression including age, stage and tumor grade. A Cox's proportional hazards model in a forward stepwise manner with the log-likelihood ratio significance test was used to select the maximum likelihood of patient survival. Probability values less than 0.05 were regarded as significant.

結果與討論

IGFBP-3 as an invasion-suppressor gene in OVTW59 cell lines.

IGFBP-3, a selected gene from our previous microarray data in invasion-suppressor cluster, exhibited higher gene and protein expressions in P0 than P4 by Q-RT-PCR and by Western blotting (Fig. 1A).

IGFBP-3 suppresses cancer cell migration, invasion and metastasis but not cell proliferation.

To determine the functional role of IGFBP-3 in ovarian cancer cell, P0 and P4 cells were stably transfected with either the pK vector (-V) or the constitutively expression vector containing *IGFBP-3* (-I). By scratch assay, we found higher migration ability in P4 cells than P0 (Figure 3B, lanes 1 and 2). Constitutive IGFBP-3 expression in P4 cells (P4-I) reduced the migration ability to the level nearly equal to P0 cells (Fig. 1B,

lanes 4 and 6). Furthermore, by suppressing the IGFBP-3 expression using RNA interference (shRNA) in P4-I cells (IGFBP-3 stable transfectant), we found the resuming of migration activity after suppression of IGFBP-3 expression (Fig. 1B, lane 8 comparing to lane 7; IGFBP-3 shRNA versus control transfection with scrambled oligo sequence). In contrast, overexpression of *IGFBP-3* did not alter the cell proliferation rate (MTT assay) in P0 and P4 transfectants (data not shown).

By transwell invasion assay, the increased invasion capability of P4 was inhibited by *IGFBP-3* transfection (Fig. 1C). In heterotransplantation, the P4-I xenograft tumors grew slower than the control P4-V. Growth arrest followed by central ulceration was observed in most P4-I xenograft tumors at around 60 days after transplantation (Fig. 1D). Metastasis occurred in all of the P4-V xenografts (4 into pleural cavities and 1 into pelvic cavity), but none in the P4-I xenografts. Histopathology of the xenografts and the metastatic tumors were similar to that of the P4 tumors (data not shown). These findings indicate that the role of *IGFBP-3* may be unique in involving ovarian EC cell migration, invasion and metastasis, but is independent of cell proliferation. Furthermore, we found minimal IGF-1 levels in P0 and P4 cells before and after IGFBP-3 transfection, suggesting that the effect of IGFBP-3 on cell migration, invasion and metastasis is not through IGF-1 inhibition, but likely is an IGF-independent effect. In addition, no changes in MMP-2 expression were found by Western blot and ELISA analyses in all IGFBP-3 transfectants (data not shown). Overall, this intriguing observation regarding IGFBP-3 suggests that other invasion related genes identified from the present experiments might have the similarly important regulatory function.

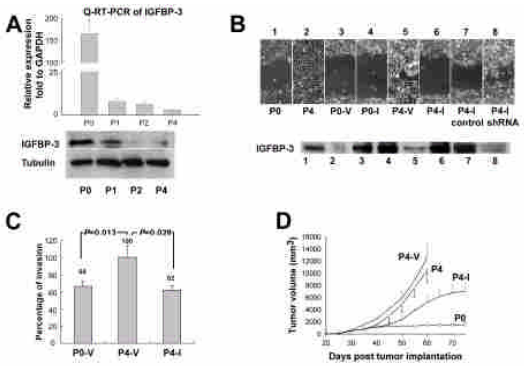

Fig 1. Functional analysis of IGFBP-3 in ovarian EC cell lines.
A: Q-RT-PCR analysis and Western blotting of IGFBP-3 in P0 to P4 cell lines. The mRNA was analyzed using GAPDH as reference. 20 μg of cell lysates were harvested for Western blot and Tubulin was used as an indicator of loading. **B**: P0-V, P0-I, P4-V, P4-I are stable P0 and P4 transfectants (-V: transfection of pK plasmid without hIGFBP-3 insert; -I: transfection of a plasmid containing hIGFBP-3 gene) (lanes 3 to

6), and lanes 7 and 8 shows transient shRNA transfectants (scrambled oligo sequence control and IGFBP-3 shRNA) or IGFBP-3 in P4-I cells. Each transfectants were incubated with DMEM with 10% FCS and their migration abilities were analyzed 17 hours after stripping. Ectopic expression of IGFBP-3 in P4 cells resulted in inhibition of cell migration (lane 6 comparing to lanes 5 and 2). Suppression of IGFBP-3 expression in P4-I cells (IGFBP-3 stable transfectant) reveals increase cell migration ability (lane 8 comparing to lane 7). Lowest lanes showed 20-fold concentrated culture mediums from the transfectants blotted for IGFBP-3 expressions. Representative data of three independent experiments using mixed stable clones are shown. *C*: invasion ability of P0 and P4 transfectants by membrane invasion culture system (MICS). Cells were seeded at $5x10^4$ cells per well, incubated for 24 hours and numbers of cells invaded through the 8 μm pore sized membrane invasion culture system (MICS) were counted. The invasion ability of P4-V cells is designated as 100% ($P = 0.07$ among the three groups by Bonferroni t-test). *D*: xenograft growth in SCID mice bearing tumors of P0, P4, and P4 transfectants. Each point represents the mean of five xenografts. ($P = 0.067$ between P0 and P4 xenograft; $P = 0.029$ between P4-I and P4-V by paired *t-test*)

IGFBP-3 inhibited cancer cell migration and invasion through ERK pathway

We studied the downstream signal transduction pathways of migration and invasion in these stable transfectants and found significant suppression of the kinase activities of extracellular signal-regulated kinase (ERK) in P0-V and P0-I cells, while P4-I showed partial suppressed pERK when compared with P4-V transfectants (Fig 2A). However, IGFBP-3 showed no remarkable relationship with Akt signal pathway. Similarly, pERK expression was again suppressed by inhibiting IGFBP-3 using shRNA (Fig. 2A). When immunohistochemical staining was performed in the xenograft tumors, we found high IGFBP-3, low pERK expressions in P4-I xenografts, and low IGFBP-3, high nuclear pERK expressions in P4-V xenografts. (Fig 2B). The migration ability of P4 cells was found inhibited by MAPK kinase-1 inhibitor PD98059 (Fig. 2C).

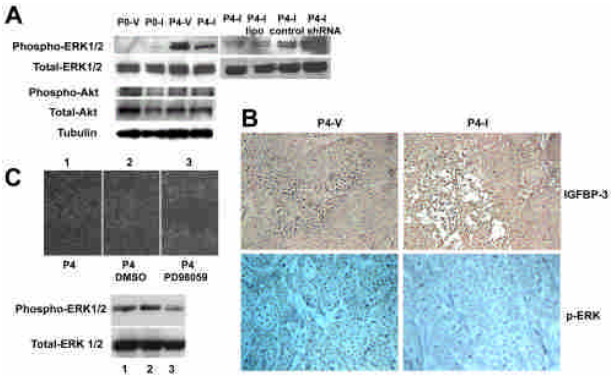

Fig 2. Signal transduction pathway of IGFBP-3 in ovarian cancer cell invasion. *A,* Transfectants were incubated with 10% serum for 24 hours and then assayed for the expressions of phosphorylation-specific ERK1/2 and Akt by Western blot. The blot was stripped and re-probed using anti-ERK, anti-Akt Ab and tubulin to serve as a loading control. *B,* Immunohistochemical staining of IGFBP-3 (upper panels) and p-Erk (lower panels) expressions in P4-V and P4-I transfectants. *C,* Scratch test showing inhibition of cell migration by 20 μM of PD98059, an MAPK kinase-1 inhibitor.

Clinical investigation showing a prognostic role of IGFBP-3 expression for patient survival

Thirty-five cases of EC were recruited and the average age of these patients was 49.4 ± 13.2 years old, where 42.8% were in the advanced stage (stages 3 and 4) and 62.9% were at higher grade (grades 2 and 3) (Table 1). Immunostaining analysis indicated that IGFBP-3 was localized to the cytoplasm in most tumor cells and expressed strongly in tumors of lower grading (Figure 3A) but weakly in higher grading (Figure 3B). Expression of IGFBP-3 was significantly associated with earlier FIGO stage and lower tumor grades. Tumor recurrence was significantly associated with stage, tumor grade and IGFBP-3 expression levels (Table 2). The mean survival time of these patients was 36.1 ± 1.9 months and the recurrence-free survival was 23.4 ± 1.5 months. The 3-year overall survival rate was 73.2% (94.1% versus 57.8% for high versus low IGFBP-3 expressions) and the 3-year recurrence-free survival rate was 61.3% (82.3% versus 35.0% for high versus low IGFBP-3 expressions). By Kaplan-Meier survival analysis, decreased IGFBP-3 expression correlated significantly with lower overall and recurrence-free survivals (Figure 3C and D). In univariate analysis, low IGFBP-3 expression correlated significantly with overall and recurrence-free survivals (Table 2). After age, but not stage and grade adjustments, IGFBP-3 was significantly correlated with overall and recurrence-free survivals. However, IGFBP-3 was found not related to patient survivals in a multivariate Cox regression analysis including age, stage and tumor grade. In stepwise selection, IGFBP-3 expression was the maximum likelihood estimate of overall patient survival, and stage for the estimate of recurrence-free survival (Table 2).

Table 1. Tumor expression of IGFBP-3 and clinocopathological features in ovarian endometrioid carcinoma (EC).

Characteristics	Patient number	Number (%) of patient with low IGFBP-3	*P* value	Number (%) of patient with recurrence	*P* value

Age					
<50 y/o	21	11 (52.3%)		9 (42.9%)	
>50 y/o	14	7 (50.0%)	0.84	4 (28.6%)	0.62
FIGO stage					
I-II	20	6 (30.0%)		3 (15.0%)	
III-IV	15	12 (66.7%)	0.01*	10 (66.7%)	0.005*
Tumor grade					
G1	13	0 (0%)		1 (7.7%)	
G2	14	11 (78.6%)		8 (57.1%)	
G3	8	7 (87.5%)	<0.001*	4 (50.0%)	0.02*
IGFBP-3 expression					
High	17	-		3 (17.6%)	
Absent/low	18	-		10 (55.5%)	0.049*
Dead of disease	8	7 (87.5%)	0.041*[#]	8 (100%)	0.003*[#]

[#] Fisher's exact test

* $P < 0.05$

Table 2. Multiple linear regression analysis of the relationships of age, stage, grade and IGFBP-3 expression to patient survival in ovarian endometrioid carcinoma (EC).

	Overall Survival			Disease-free survival		
	Coefficient (SEM)	*P*	Hazard Ratio	Coefficient (SEM)	*P*	Hazard Ratio
Univariate model						
Age	-0.040 (0.03)	0.21	0.96	-0.04 (0.03)	0.12	0.96
Stage	0.98 (0.40)	0.015*	2.66	0.75 (0.28)	0.008*	2.11
Grade	0.75 (0.45)	0.10	2.11	0.79 (0.36)	0.026*	2.21
Low IGFBP-3	2.35 (1.07)	0.028*	10.49	1.62 (0.67)	0.016*	5.06
Two covariate model						
Low IGFBP-3	2.34 (1.08)	0.03*	10.38	1.78 (0.69)	0.001*	5.92
Age	-0.04 (0.03)	0.21	0.96	-0.05 (0.03)	0.08	0.95
Low IGFBP-3	1.73 (1.17)	0.14	5.61	1.03 (0.81)	0.20	2.80
Stage	0.06 (0.44)	0.17	1.84	0.47 (0.34)	0.16	1.60

Low IGFBP-3	2.67 (1.39)	0.05*	14.50	1.25 (0.92)	0.17	3.50
Grade	-0.27 (0.74)	0.71	0.76	0.30 (0.53)	0.57	1.36
Multivariate analysis model						
Age	-0.06 (0.04)	0.08	0.94	-0.10 (0.03)	0.01*	0.91
Stage	0.99 (0.48)	0.08	2.69	1.03 (0.46)	0.02*	2.81
Grade	0.51 (0.90)	0.57	1.66	1.04 (0.58)	0.07	2.82
Low IGFBP-3	1.04 (1.31)	0.43	2.83	0.06 (0.87)	0.94	1.07
Stepwise selection						
Low IGFBP-3	2.35 (1.07)	0.03*	10.49			
Stage				0.75 (0.28)	0.008*	2.11

SEM: standard error of mean

* $P < 0.05$

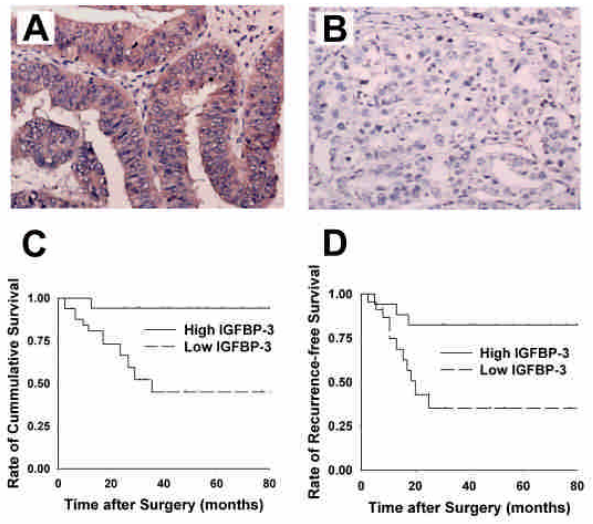

Fig 3. Functional analysis of IGFBP-3 in ovarian tissues. *A* and *B*: paraffin sections showing IGFBP-3 immunohistochemical staining in a grade 1 (A) and a grade 3 (B) EC tumor (x 200). *C* and *D*: Kaplan-Meier survival analysis according to the degree of IGFBP-3 expression in EC patients. Overall (C) and recurrence-free (D) patient survival rates in tumors with lower (dash line) or higher (black line) expressions of IGFBP-3 (both $P < 0.007$).

Conclusion: We identified IGFBP-3 as a potential invasion suppressor gene in ovarian endometrioid carcinoma through down regulation of ERK. The fact that IGFBP-3 expression is suppressed in high-grade carcinoma and correlated with survival in ovarian endometrioid carcinoma underlines its important role in tumor

progression.

Reference:

1. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocine Rev 2002:23;824-54.
2. Han VK, Lund PK, Lee DC, D'Ercole AJ. Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. J Clin Endocrinol Metab 1988;66:422-9.
3. Macaulay VM. Insulin-like growth factors and cancer. Br J Cancer 1992;65:311-20.
4. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. Plasma insulin-like growth factor-I and prostate cancer risk:prospective study. Science 1998;279:563-6.
5. Hankinson SE, Willett WE, Colditz GA, Hunter DJ, Michaud DS Deroo B Rosner B, Speizer FE, Pollak M. Circulating concentration of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393-6.
6. Shimisaki S, Ling N. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5, and -6). Prog. Growth Factor Res 1991;3:243-266
7. McCusker RH, Busby WH, Dehoff MH, Camacho-Hubner C, Clemmons DR. Insulin-like growth factor (IGF) binding to cell monolayers is directly modulated by the addition of IGF-binding proteins. Endocrinology 1991;129**:**939-949
8. H. Yu and T. Rohan. Role of the Insulin-Like Growth Factor Family in Cancer Development and Progression. J Natl Cancer Inst 2000;92:1472-89.
9. Hendrix MJ, Seftor EA, Seftor RE, Fidler IJ. A simple quantitative assay for studying the invasive potential of high and low human metastatic variants. Cancer Lett 1987;38:137-47.
10. Valentinis B, Bhala A, DeAngelis T, Baserga R, Cohen P. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. Mol Endocrinol 1995;9:361-7.
11. Torng PL, Mao TL, Chan WY, Huang SC, Lin CT. Prognostic significance of stromal metalloproteinase-2 in ovarian adenocarcinoma and its relation to carcinoma progression. Gynecol Oncol 2004;92:559-67.