鈷氯離子共同輸送體在子宮頸癌致癌機轉中角色之研究

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-006-021-
執行期間：93年08月01日至94年07月31日
執行單位：國立成功大學醫學系婦產科

計畫主持人：周振陽

報告類型：精簡報告
處理方式：本計畫可公開查詢

中華民國94年6月1日
行政院國家科學委員會專題研究計畫成果報告

鉀氯離子共同輸送體在子宮頸癌致病機轉中角色之研究：(2/3)

計畫編號：NSC 93-2314-B-006-021
執行期限：93年8月1日至94年7月31日
主持人：周振陽　　執行機構及單位名稱：成大醫學院婦產科

一. 中文摘要

本研究主要探討對 IGF-1 接受器的訊息傳遞系統在子宮頸癌形成及轉移所扮演的角色。研究結果發現可明顯促進子宮頸癌細胞的生長及增加細胞的侵襲性。而上述的作用可以完全被接受器抗體所抑制。另外，在早期子宮頸癌病人中，的表現和子宮頸癌的旁組織或淋巴結轉移有高度相關，更重要的是的高度表現的病人其預後比起不表現的病人明顯較差，本研究結果顯示訊息傳遞在未來可作為預防或治療子宮頸癌的一種方法。

二. Summary

This study is to test the hypothesis that IGF-1 growth factor receptor signalings may functionally promote cervical cancer development. The results demonstrate that insulin-like growth factor 1 (IGF-1) is a potent stimulator of cervical cancer cell invasion and proliferation. The IGF-1-stimulating effects are completely inhibited by antagonist antibody against IGF-1 receptor (IGF-1R). Furthermore, among the 71 patients with early-stage cervical cancer it was found that IGF-1R overexpression was more frequently detected in cervical cancer cells with a strong tendency to invade or metastasize, and was associated significantly with poor patient survival. Thus, blockade of IGF-1R signalings may provide novel strategies for the treatment of invasive phenotypes of cervical cancer.

三. Introduction

Cervical cancer is the second leading cause of cancer death in women worldwide. There are large differences in incidence rates of invasive cervical cancer among populations, reflecting the influence of environmental factors, screening Pap smears, and the treatment of pre-invasive lesions. A growing body of evidence has accumulated to indicate that oncogenic types of human papillomavirus (HPV) serve as an important factor in the development of the precursors of cervical cancer. The protein products of HPV DNA appear to be interactive with the antioncogenic function of retinoblastoma gene and p53 gene. The pathogenesis of cervical cancer shows multiple stages of progression, from HPV-related cellular growth dysregulation to cervical cancer. However, only a small fraction of those infected by HPV develop cancer, indicating that other factors contribute to the progression to cervical cancer. Despite intensive studies that have been carried out, the tumor biology of this disease is still largely unknown.

The spread of malignant tumor cells to secondary sites imposes a serious problem in the prognosis and treatment of neoplastic disease. Although prognostic factors such as parametrium invasion and lymph node metastasis affect the outcome of cervical cancer, the variability in progression-free and overall survival among patients with similar clinical and pathological characteristics makes it difficult to predict the outcome reliably. Adhesion receptors of the integrin family promote cell attachment to proteins within the extracellular matrix and potentiate cellular migration and invasion. A correlation has been established between specific integrins and metastatic behavior in vivo. Tumor cells can secrete cytokines such as growth factors, which may lead to autocrine stimulation of tumor cell growth and/or motility. Thus, it appears that the interaction of growth factors and adhesion proteins likely contributes to the metastatic and invasive cascades. For example, IGF-1 induces adhesion and migration in human multiple myeloma cells via activation of β1 integrin signalings. In breast cancer MCF-7 cell line, IGF-1-triggered cellular migration and invasion are mediated by αvβ3 integrin. Integrin αvβ3 and αvβ1 are the key regulators of hepatocarcinoma cell invasion across the fibrotic matrix microenvironment in response to the stimulation of basic fibroblast growth factor and epidermal growth factor (EGF).

Little is known about how the molecular events of integrin and growth factor receptor regulate the proliferation and invasion of cervical cancer cells. This study is aimed to identify the specific growth factors which are critically involved in cervical cancer cell invasion and proliferation, and test the...
hypothesis that growth factor receptor signalings may cooperate functionally to promote cancer development and progression.

IV. Results

IGF-1 is a potent stimulator of invasive migration. The invasion assays were performed in cervical cancer SiHa and CaSki cells which have been incubated with different concentrations of various growth factors for 24 hours. As shown in Figure 1A & 1B, SiHa and CaSki cells migrated in response to the stimulation of IGF-1, EGF and transforming growth factor beta (TGFβ). Dose-response assays done with both cell types established that 50 ng/ml IGF-1 was an optimal concentration for maximal stimulation of tumor invasion (4.4±0.3-fold & 4.2±0.4-fold increase compared with DMEM/0.1% FCS alone for SiHa and CaSki cells, respectively), when fibronectin was used as chemoattract in the lower Boyden’s chamber. We further compared the invasive migration between normal cervical epithelial cells and cervical cancer cells. Cervical cancer SiHa and CaSki cells presented the better capability of invasion than that of normal epithelial cells in the absence of any stimulation of growth factors (Figure 1C & 1D). Although IGF-1 modestly stimulated the invasion of normal epithelial cells, the invasive capability of SiHa and CaSki cells was markedly enhanced by IGF-1 stimulation when fibronectin or vitronectin was used as chemoattract (Figure 1C & 1D). This indicates that IGF-1 can differentially enhance the invasive migration between cervical cancer cells and normal cervical epithelial cells. Furthermore, IGF-1-stimulated invasion was completely inhibited by functional-blocking monoclonal antibody against α-subunit of IGF-1R in SiHa and CaSki cells (Figure 2A & 2B), whereas the invasion was unaffected by either IgG or monoclonal antibody to IR.

IGF-1 stimulates cervical cancer cell growth. We studied cell proliferation under various culture conditions, to test whether the growth of cervical cancer cells is regulated by IGF-1. As shown in Figure 3A, the growth of cervical cancer cells (SiHa and CaSki) was stimulated dose-dependently by IGF-1, whereas that of normal cervical epithelial cells was not. In addition, the antagonistic monoclonal antibody of IGF-1R, but not IR and IgG, abolished IGF-1-stimulated cancer growth (Figure 3B). Figure 3C shows the effect of blocking antibodies of IGF-1R on Annexin V binding, as assessed by fluorescence-activated cell sorter analysis. Pictured by the increase in Annexin V binding, IGF-1R blocking antibodies induced an increase in externalization of phosphatidyl serine from the inner leaflet of the plasma membrane, a typical early sign for apoptotic cell death. This indicates that IGF-1R mediate the survival signalings that inhibit apoptosis.

Patterns of IGF-1, IGF-1R expression in cervical cancer tissues. Studies in cell culture systems have revealed that IGF-1 and IGF-1R signalings are important modulators of cervical cancer cell proliferation and invasion. To test the in vivo condition, we examined the expression of IGF-1 and IGF-1R in surgical specimens (n=71) of cervical cancer by immunofluorescent staining. To compare the IGF-1/IGF-1R expression in the different phenotypes of cervical cancer (these surgical specimens), we define the invasive phenotype of cervical cancer as tumors invading the parametrium or metastasizing to pelvic lymph nodes. In contrast, both the parametrium and pelvic lymph nodes are free of tumor in the non-invasive type of cervical cancer. IGF-1 protein was nearly undetectable in normal or noncancerous cervical tissues of all surgical specimens examined (Figure 7B, n=71). In contrast, adjacent cervical cancerous tissues clearly expressed IGF-1 protein. Furthermore, the distribution and intensity of IGF-1 expression were different between non-invasive and invasive phenotypes of cervical cancer (Figure 7B). More than 50% of cancer tissues in the invasive types of cervical cancer displayed strong IGF-1 stain. In contrast, the non-invasive phenotype of cervical cancer tissues showed weak IGF-1 stain. The strong IGF-1 stain was also noted in the tumor-invaded parametrium and tumor-metastasized pelvic lymph node (Figure 7B). On the other hand, no IGF-1 stain was found in the tumor-free parametrium and pelvic lymph node. The double stains identify the colocalization of IGF-1 and IGF-1R in cancer tissues (Figure 7C), suggesting autocrine or paracrine stimulation of tumor growth and invasion.

IGF-1R overexpression is a poor prognostic factor. We analyzed the clinical outcome of cervical cancer patients by IGF-1R expression. Table 1 shows the clinicopathological characteristics of cervical cancer patients grouped by the grade of IGF-1R stain. Group 1 includes the grades 1 and 2 of IGF-1R expression which indicates IGF-1R staining intensity and distribution are less than 50% of tumor area. Group 2 indicates the grades 3 and 4 of IGF-1R expression which shows IGF-1R staining intensity and distribution are more than 50% of tumor area. Tumor with strong staining of IGF-1R (Group 2) presents significantly higher percentage of parametrium invasion, pelvic lymph node metastasis and larger tumor size (Table 1). Most importantly, there were significant differences in the distributions of disease-free survival and overall survival according to the presence or absence of IGF-1R overexpression (Figure 9, P<0.005 for both comparison).

Discussion

This study clearly demonstrates that IGF-1 is a potent stimulator of cervical cancer cell invasion and proliferation. The IGF-1-stimulating effects are completely inhibited by antagonistic antibody against IGF-1R, whereas IgG or monoclonal antibody to IR has no effect. This indicates that IGF-1-stimulating effects are specifically mediated through IGF-1R
signalings. More importantly, IGF-1 can differentially enhance the invasion and proliferation between cervical cancer cells and normal cervical epithelial cells. Our experiments also show that blockade of IGF1-R occupancy induces apoptosis of tumor cells. This finding is consistent with the proposed antiapoptotic role of IGF1-R signalings (21, 22).

The immunofluorescent studies on surgical specimens further support the notion that IGF-1/IGF1-R pathway contributes to a more aggressive malignant phenotype of cervical cancer. Cervical cancer tissues overexpress both IGF-1 and IGF-1R, as compared with normal or non-cancerous cervical tissue. In addition, IGF-1 and IGF-1R colocalize in cancer tissues, supporting the view that an autocrine or paracrine interaction between IGF1-R and its ligands regulates cell proliferation and invasion. Cervical cancer cells with a strong tendency to invade or metastasize have higher expression of IGF-1/IGF1-R than those with a low ability to do so. Most importantly, IGF-1R overexpression is associated significantly with poor patient survival.

Take together, this study identifies the IGF-1R/PI3K/Akt pathway contributes to a more aggressive malignant phenotype of cervical cancers (summarized in Figure 11). Antagonists of IGF-1R signal transduction may provide novel strategies for the treatment of invasive types of cervical cancer.

六. References

20. Ling, Y., Maile, L.A., and Clemons, D.R. Tyrosine phosphorylation of the beta3-subunit of the alphaVbeta3 integrin is required for membrane association of the tyrosine phosphatase SHP-2 and


七、計劃導致的著作
Shen MR, CY Chou, KF Hsu, HS Liu, PB. Dunham, EJ. Holtzman, JC Ellory. Insulin-like growth factor 1 receptor cooperates with αvβ3 integrin to promote cervical cancer development and progression (submitted)