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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※ 研究一氧化氮與環氧化二酶對缺氧子宮頸癌細胞株之影響並與臨床預後做相關性探討※ ※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫 □整合型計畫
計畫編號：NSC92－2314－B－006－057－
執行期間：92 年 8 月 1 日至 93 年 7 月 31 日

計畫主持人：陳海雯

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執行單位：國立成功大學醫學院放射線學科

中華民國 93 年 9 月 16 日
行政院國家科學委員會專題研究計畫成果報告
研究一氧化氮與環氧化二酶對缺氧子宮頸癌細胞株之影響
並與臨床預後做相關性探討
Exploring the role of nitric oxide and cyclo-oxygenase 2 in irradiating hypoxic cervical cell lines and correlating with clinical outcome of cervical cancer patients treated by radiotherapy

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一、中文摘要
目的：研究缺氧和有氧狀況下的人類子宮頸癌細胞株（HT3 和 SiHa）的輻射敏感性，與一氧化氮（NO）之間的關係，並研究一氧化氮誘發合成酶（iNOS）、環氧化二酶（COX-2）的表現與對子宮頸癌病人接受放射線治療的臨床治療成果之影響。

材料與方法：本計畫先針對人類子宮頸癌細胞株（HT3 和 SiHa）在有氧和缺氧狀態下測量其輻射敏感性，將細胞株置於 0, 1, 2, 3, 5 和 7 格雷的放射線劑量下照射，再依其存活的細胞菌落分析其存活率。以加入藥劑 S-nitroso-N-acetyl-D,L-penicillamine（SNAP）來產生一氧化氮，細胞分別在有氧和缺氧的狀況下接受放射線照射，並加入 SNAP 後再以同樣的劑量照射，分析其細胞存活率是否有差異。病人方面則以自 1989 年到 2003 年在本院接受放射線治療的 173 人為材料，以免疫組織學的染色方法研究腫瘤細胞在 iNOS 和 COX-2 腫瘤標記的差異，再佐以比較病人的疾病局部控制存活率、無疾病存活率和全部存活率做比較。

結果：子宮頸癌細胞株 HT3 和 SiHa 在缺氧狀況下，有較強的輻射抵抗性（即較不輻射敏感）。加入能產生一氧化氮的藥劑 SNAP 後，能將缺氧狀況下的輻射敏感性大為提昇到和有氧狀況下一樣。iNOS 和 COX-2 的陽性率分別為 58.4% 和 62.4%。iNOS 和 COX-2 彼此間的表現呈現正相關（Spearman 氏相關係數 = 0.54, P < 0.01）。同時表現 iNOS 和 COX-2 者為預測是否會遠處轉移的獨立因子（OR = 5.97 和 10.20，兩者 P 值皆 < 0.01），iNOS 和 COX-2 表達量高者有顯著較差的無疾病存活率（兩者 P < 0.01）和全部存活率（P 值分別為 0.03 和 0.02）。同時高強度表現 iNOS 和 COX-2 的病人有最差的存活率，多變數分析顯示 iNOS 和 COX-2 同時達質、有較大之腫瘤、較晚期之疾病，年齡小於 50 歲和非鱗狀上皮細胞型態者都是對無疾病存活率有有意義的預後因子。

結論：一氧化氮可以作為促進缺氧子宮頸癌細胞株的輻射敏感增進劑，同時表現 iNOS 和 COX-2 的子宮頸癌病人接受放射線治療後有最差的無疾病存活率，iNOS 和 COX-2 也許可以成為預測欲接受放射線治療的子宮頸癌病人之臨床治療成果有用的生物標記。

關鍵詞：子宮頸癌，一氧化氮，COX-2，缺氧，放射治療
Abstract

**Purpose:** To study the radiosensitivity of human cervical cancer cell lines HT3 and SiHa, and investigate the impact of nitric oxide (NO) on these cells in aerobic and hypoxic conditions to ionizing radiation. Also to investigate the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in cervical cancer and their association with clinical outcome in patients treated with radical radiotherapy.

**Methods and Materials:** Radiosensitivity of two human cervical cancer cells HT3 and SiHa in aerobic and hypoxic conditions were studied by colony formation assay following ionizing radiation of 0, 1, 2, 3, 5, 7 Gy. Effects of NO on radiosensitivity of HT3 and SiHa in both aerobic and hypoxic conditions were assessed by adding SNAP (NO-releasing agent S-nitroso-N-acetyld,L-penicillamine) to cell cultures and by colony formation assay. One hundred seventy-three consecutive patients with FIGO Stages IB to IVA cervical cancer underwent radical radiotherapy, including external-beam radiotherapy and/or high-dose-rate brachytherapy between 1989 and 2002. Immunohistochemical studies of their formalin-fixed, paraffin-embedded tissues were performed. Univariate and multivariate analyses were performed to identify and evaluate the effects of the factors affecting patient survival.

**Results:** HT3 and SiHa were more radioresistant in hypoxic conditions. This radioresistance could be reverted by adding SNAP into cell cultures. Positive immunostainings of iNOS and COX-2 were observed in 58.4% and 62.4% of the participants, respectively. iNOS and COX-2 expressions were positively correlated (Spearman correlation coefficient = 0.54, \( P < 0.01 \)), and their overexpressions were independent predictors of distant metastasis (OR = 5.97 and 10.20, respectively; \( P < 0.01 \) for all). iNOS- and COX-2-expressing patients had significantly shorter disease-free survival (DFS) (\( P < 0.01 \), both) and cause-specific overall survival (OS) (\( P = 0.03, P = 0.02 \), respectively). Patients with iNOS-positive/COX-2-positive tumors had the poorest survival rates. iNOS/COX-2 co-expression, together with bulky tumor, advanced stage, age < 50 years, and non-squamous cell histology, were independent prognostic factors for DFS.

**Conclusions:** NO could enhance radiosensitivity of cervical cancer cell lines in hypoxic condition. Overexpression of iNOS and COX-2 were associated with decreased survival in cervical cancer patients treated with radiotherapy. Co-expression of iNOS and COX-2 may represent useful biologic markers in patients receiving radical radiotherapy for cervical cancer.

**Keywords:** Cervix cancer, nitric oxide, COX-2, hypoxia, radiotherapy.

二、研究成果報告

**Introduction:**

Nitric oxide (NO) is a potent biological molecule that mediates a diverse array of activities, including vasodilatation, neurotransmission, and immune defense (1). Increasing evidence suggests that increased NO production by tumor cells plays a critical role in cancer development. The iNOS enzyme has been implicated in carcinogenesis (2), tumor angiogenesis and cancer progression (3), and metastasis (4). Studies have shown increased expression of iNOS in several types of gynecological cancer (5). But the prognostic role of iNOS in cervical cancer still remains unclear.

On the other hand, much attention has been focused on the biological and clinical role of cyclooxygenase-2 (COX-2), one of the two isoforms of the key enzyme in the conversion of arachidonic acid to prostaglandins, in the pathogenesis and natural history of cervical cancer. COX-2 expression is increased in more severe grades of cervical dysplasias and invasive cervical cancer (6). High COX-2 expression is also associated with lymph node or parametrial involvement in cervical tumors (7). Moreover, studies in small series of patients have shown that elevated COX-2 expression correlates with diminished survival and resistance to...
chemotherapy in cervical cancer patients (8, 9).

NO can promote tumor progression by stimulating COX-2. Concentrations of both NO and prostaglandins have been positively associated with tumor progression. The efficacy of tumor response to irradiation has been increased by NO and COX-2 inhibitors (10, 11). Although some studies have shown COX-2 as an independent prognostic indicator in cervical cancer patients, the prognostic significance of iNOS and the co-expression of iNOS and COX-2 has not been investigated.

The aim of the present study was to study the radiosensitivity of human cervical cancer cell lines HT3 and SiHa, and investigate the impact of NO on these cells in aerobic and hypoxic conditions to ionizing radiation. We also would like to investigate the expression of iNOS and COX-2 by immunohistochemistry and their association with clinicopathologic parameters and clinical outcome in a large single institutional series of patients treated with radiotherapy.

Material and methods:

Cell culture

Two human cervical cancer cells HT3 and SiHa were grown in 10% fetal bovine serum-supplemented Dulbecco’s modified Eagle’s medium (FBS-DMEM) with penicillin (100 units/ml) and streptomycin (0.1 mg/ml).

Irradiation and colon formation

Cells in exponential growth were irradiated using 6MV photon beams by a Linac. Radiosenstivity of HT3 and SiHa were studied by colony formation assay following ionizing radiation of 0, 1, 2, 3, 5 and 7 Gy. Colonies of at least 50 cells were scored as surviving cells. For incubation in various concentrations of O2, 90% confluent cell cultures in 100-mm plates were placed either in a standard carbon dioxide (CO2) incubator (5% CO2 in air) or in airtight chambers that were flushed with a 5% CO2-95% nitrogen mixture. O2 concentrations within these chambers were maintained at specified levels using Pro-OxO2 regulators.

Patients

The study included 173 International Federation of Gynecology and Obstetrics (FIGO) stage IB to IVA patients (age range: 26-89; median: 68) with cervical cancer for whom tissue blocks were available and who were consecutively treated in our hospital’s Department of Radiation Oncology between 1989 and 2002.

Results:

- Radiosensitivity of HT3 and SiHa in anobic(20% O2) and hypoxic conditions (1% O2)

- Radiosensitivity of SiHa with and without NO donors in anobic(20% O2) and hypoxic (1% O2) conditions
- **iNOS Immunostaining**

- **COX-2 Immunostaining**

- **Disease-free survival (DFS) curves in cervical carcinoma patients with iNOS-positive tumors and iNOS-negative tumors**

- **The combination of iNOS and COX-2 expressions and its impact on disease-free survival (DFS)**

- **Disease-free survival (DFS) curves in cervical carcinoma patients with COX2-positive tumors and COX2-negative tumors**

- **The combination of iNOS and COX-2 expressions and its impact on cause-specific overall survival (OS) rates**

**Conclusions:**
NO could enhance radiosensitivity of cervical cancer cell lines in hypoxic condition. Overexpression of iNOS and COX-2 were associated with decreased survival in cervical cancer patients treated with radiotherapy. Co-expression of iNOS and COX-2 may represent useful biologic markers in patients receiving radical radiotherapy for cervical cancer.
五、參考文獻


