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Expression of EGFR (Epidermal Growth Factor Receptor) and C-KIT in Nasopharyngeal Carcinoma

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Abstract Nasopharyngeal carcinoma (NPC) is the most common head and neck cancers in Thailand. Although several genetic changes related to NPC have been identified, radiation or chemoradiation still plays an important role in the treatment. The recent development of new anti-cancer agents directed at specific signaling pathways has prompted the researchers to examine the expression of EGFR (epidermal growth factor receptor) and C-KIT (CD117) in Thai patients with NPC. Immunohistochemical method was used to evaluate expression of the proteins in 55 NPC samples retrieved from pathology files at the Institute of Pathology, Department of Medical Services and the Department of Pathology, Faculty of Medicine, Chulalongkorn University during 2003-2004. Fifty-two (95%) and twelve (22%) cases were immunoreactive with EGFR and C-KIT, respectively. No correlation was found between expressions of the two markers. NPC patients with positive specific signaling protein(s) may be eligible for the new anti-cancer treatments, but further studies are needed to determine the actual benefit of such medications.

Key words: **nasopharyngeal carcinoma, EGFR, C-KIT**

Introduction

Nasopharyngeal carcinoma (NPC) is the most frequently found head and neck cancers in Thailand, with age-standardized rate of 4.5 and 1.6 per 100,000 male and female populations, respectively.[1] Association between the cancer and Epstein-Barr virus (EBV) is well-documented, and several genetic alterations have been implicated in NPC tumorigenesis.[2,3] More recently, EBV has been detected in serum of NPC patients, and the circulating viral DNA has subsequently been found to be a useful indicator for the response to treat-

ment.[4,5] Despite the effort to unravel molecular changes, which underlie NPC, and the sensitive tumor marker, radiation or chemoradiation is still the primary mode of therapy.

In the recent years, new treatments of cancer directed at specific signaling pathways have received particular attention; for example, imatinib for C-KIT-positive gastrointestinal stromal tumor,[6] and gefitinib for pulmonary adenocarcinoma with mutations of the EGFR (epidermal growth factor receptor) gene.[7] Both EGFR and C-KIT are transmembrane receptors, activation of which are related to oncogenesis and progression of some cancers.[6,7] The primary objective of this study was to evaluate C-KIT and EGFR expression in Thai NPC patients. The minor objective was to determine the possible association of the two proteins in NPC.

Methodology

Cases of nasopharyngeal carcinoma (NPC) were retrieved from pathology files at the Institute of Pathology and the King Chulalongkorn Memorial Hospital during 2003-2004. Biopsies performed after treatment and specimens containing a limited amount of cancer cells were not included. After exclusion, 55 NPC samples were enrolled on this study which was carried out from January 2005 to August 2005.

All samples were reviewed and classified according to the World Health Organization classification (WHO) of NPC.[8] Keratinized NPC (WHO I) is characterized by well-defined sheets of cancer cells, with presence of intercellular bridges and/or keratin production. Well-defined sheeting architecture is also the feature of non-keratinized NPC (WHO II) but no intercellular bridges or keratinization is observed. Undifferentiated carcinoma of the nasopharynx (WHO III) consists of poorly-formed sheeting pattern. Large pleomorphic tumor cells are typically admixed with a number of lymphocytes.

Three-micrometer-thick tissue sections were deparaffinized, then rehydrated in graded alcohols, and rinsed in distilled water. Detection of EGFR (monoclonal, dilution of 1: 100) and C-KIT (polyclonal, dilution of 1: 1000) was performed, using standard indirect immunohistochemical staining method. Both primary antibodies were obtained from DakoCytomation, CA, USA. Immunostained slides were evaluated by 2 pathologists. Membrane staining for EGFR, and membrane and/or cytoplasmic staining for C-KIT, were semi-qualitatively counted. The scoring included percentage of immunoreactive tumor cells, which was graded as 0 (negative) to 4+ (less than 5%, 6-25%, 26-50%, 51-75%, and 76-100% tumor cells, respectively). Correlation between EGFR and C-KIT expressions was determined by Chi-square or Fisher exact test when applicable. P value of less than 0.05 was considered significant.

Results

The present NPC cohort included 43 male and 12 female patients, with the age ranging from 23-86 (mean = 49.8) years. The majority was WHO III (46 cases). WHO I NPC was found in 1 case, WHO II NPC in 4 cases, and mixed WHO II and III NPC in the remaining (4 cases). The majority of NPC (52 cases) expressed EGFR (95%) (figure 1), whereas only 22 percent (12 cases) were positive for C-KIT (figure 2 and table 1). No correlation was found between expressions of the two proteins (Fisher exact test, p = 0.12) (Table 2).

Discussion

Epidermal growth factor receptor (EGFR) or HER1 is transmembrane receptor encoded by

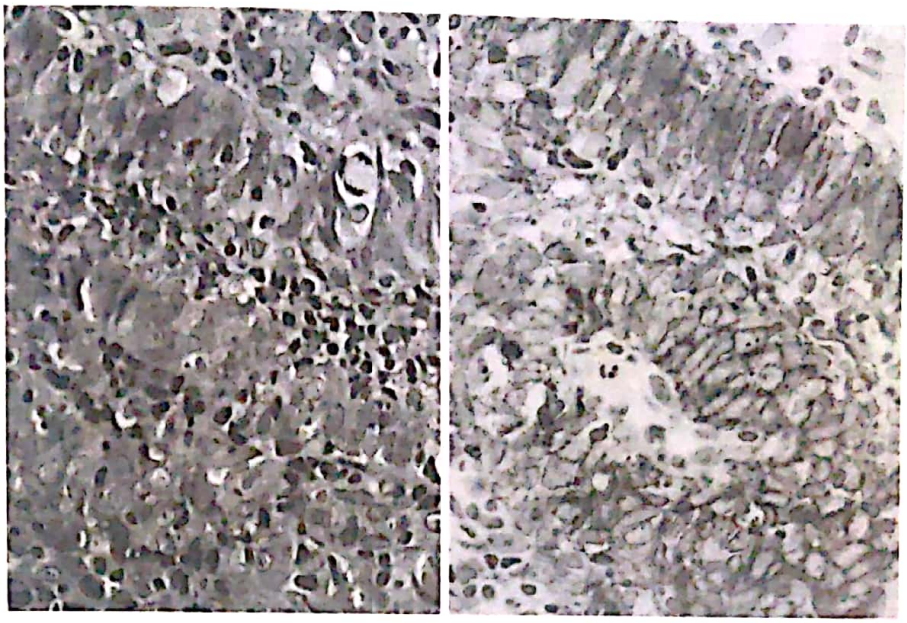

Figure 1 Expression of EGFR in Nasopharyngeal carcinoma (Left = H&E stain, Right = EGFR immunostain).

chromosome 7.[9] Ligand binding of the EGF receptor activates the EGFR tyrosine kinase, resulting in cell growth and differentiation. EGF receptor cascades may also promote malignant transformation, angiogenesis, and/or metastatic dissemination.[9] The extent of EGFR expression has been found to have impact on survival and locoregional recurrence of colorectal carcinoma.[9,10] Recently , gefitinib (anti-EGFR agent) has been shown to be effective for patients with pulmonary adenocarcinoma harboring EGFR mutations.[7]

C-KIT or CD117 is a transmembrane tyrosine kinase receptor in which the extracellular portion binds a ligand recognized as stem-cell factor and the intracellular portion contains the actual kinase enzymatic domain.[11] A gain-of-function mutation in the C-KIT proto-oncogene is a central tumori-

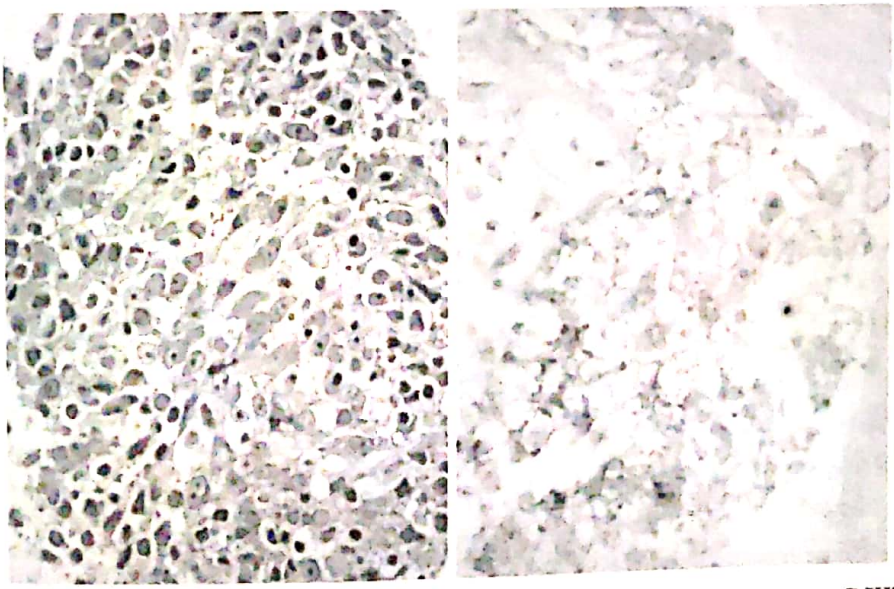

Figure 2 Expression of C-KIT in Nasopharyngeal carcinoma (Left = H&E stain, Right = C-KIT immunostain).

Table 1 Expression of EGFR and C-KIT in nasopharyngeal carcinoma

	Negative	Positive				Total
		+	++	+++	++++	
EGFR	3	5	14	19	14	55
C-KIT	43	7	5	0	0	55

Table 2 Correlation between EFGR and C-KIT in nasopharyngeal carcinoma

	C-KIT-positive NPC	C-KIT-negative NPC
EGFR-positive NPC	10	42
EGFR-negative NPC	2	1

Fisher exact test, p = 0.12

geal carcinoma in the current cohort expresses EGFR, but the minority does so with C-KIT. No correlation was found between expressions of the two proteins. NPC patients, with tumor cells positive for any of the markers, may be beneficial with anti-CD117 or anti-EGFR agents. However, the actual value of the treatments in NPC requires further prospective studies.

genic event in gastrointestinal stromal tumor (GIST). STI-571 (imatinib) has proven effective in metastatic GIST, and provided proof of the theory that a specific molecular inhibitor can drastically and selectively change the survival of tumor cells with a particular genetic alteration.[6]

With regard to nasopharyngeal carcinoma (NPC), although expression of EGFR and C-KIT has been determined, the possible relationship of the two proteins in NPC has not been explored. Furthermore, no data are available regarding the EGFR and C-KIT expression in Thai patients with NPC. In line with the previous studies, EGFR expression was found in the majority (95%) of NPC. The frequency of C-KIT expression was slightly lower (22% compared to 33%),[12] but EGFR was higher (95% compared to 82-84%)[13,14] than those observed in the previous reports. No correlation was found between EGFR and C-KIT immunoreactivity (Fisher exact test, p = 0.12).

In conclusion, the majority of nasopharyn-

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บทคัดย่อ **การแสดงออกของ EGFR และ C-KIT ในมะเร็งหลังโพรงจมูก**

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มะเร็งหลังโพรงจมูกเป็นมะเร็งของศีรษะและคอที่พบได้บ่อยที่สุดในประเทศ แม้ว่าจะมีการตรวจพบความผิดปกติต่าง ๆ ทางพันธุกรรมของมะเร็งชนิดนี้ การรักษาหลักที่ใช้อยู่แพร่หลายในปัจจุบัน ยังคงเป็นการให้รังสีรักษา หรือเคมีบำบัดร่วมกับรังสีรักษา เมื่อไม่นานมานี้ได้มีการพัฒนายาต้านมะเร็งชนิดใหม่ ๆ ที่ออกฤทธิ์โดยตรงต่อ signaling pathways คณะผู้วิจัยจึงได้ทำการศึกษาการแสดงออกของ EGFR (epidermal growth factor receptor) และ C-KIT (CD117) โดยวิธีอิมมูโนฮีสโตเคมี ในมะเร็งหลังโพรงจมูกของผู้ป่วยชาวไทย ที่มีประวัติและตัวอย่างในสถาบันพยาธิวิทยา กรมการแพทย์ และภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในระหว่าง พ.ศ. ๒๕๔๖ ถึง ๒๕๔๗ ผลการศึกษามะเร็งหลังโพรงจมูกจำนวน ๕๕ ราย พบการแสดงออกของ EGFR และ C-KIT จำนวน ๕๒ ราย (ร้อยละ ๙๕) และ ๑๒ ราย (ร้อยละ ๒๒) ตามลำดับ ไม่พบความสัมพันธ์ระหว่างการแสดงออกของโปรตีนทั้งสองชนิด ผู้ป่วยมะเร็งหลังโพรงจมูกที่เซลล์เนื้องอกให้ผลบวกต่อโปรตีนดังกล่าวอาจมีการตอบสนองต่อยาต้านมะเร็งชนิดใหม่ ๆ แต่ผลลัพธ์ที่แน่นอนต้องศึกษาต่อไป

คำสำคัญ: **มะเร็งหลังโพรงจมูก, EGFR, C-KIT**