行政院國家科學委員會專題研究計畫 成果報告

接受器酪酸激酶 与 同時表現或 表現型的改變在
判斷大腸直腸癌病人預後的角色

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※ 接受器酪酸激脢 RON 與 MET 同時表現
或 RON 表現型的改變在判斷大腸直腸
癌病人預後的角色（94-2314-B-006-058-）(2/2) ※

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□ 國際合作研究計畫國外研究報告書一份

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Prognostic Significance of Co-expression of RON and MET Receptors in Colorectal Cancer Patients

計畫編號：NSC 94-2314-B-006-058

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1. Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the Western world and the third one in Taiwan. At present, CRC is treated by a narrow range of options including surgical resection, radiotherapy and 5-fluorouracil (5-FU) and folinic acid chemotherapy. Despite curative surgery, approximately 40% of patients still have disease relapse leading to morbidity and eventual mortality. Hence, there is a need to find new molecular targets for therapy and identify which of these patients would benefit from adjuvant therapy. In CRC, a limited number of predictive markers have been identified and used to date, but the results are somewhat inconclusive (Salonga et al, 2000; Takebe et al, 2001). However, the use of these predictive markers individually has led to somewhat inconclusive results. (Allen et al, 2005).

Protein tyrosine kinases (PTKs) are a major class of protooncogenes and play a crucial role in many cell regulatory processes, such as proliferation, migration, adhesion, and, potentially, cellular transformation. Currently, most of the established proto-oncogenes in solid tumours are PTKs. MET (HGF receptor) tyrosine kinase, epidermal growth factor (EGF) receptor, and ErbB2 (c-erbB-2, HER-2/neu) are well-known examples. The involvement of MET in human tumors has been definitively established and can be achieved through several mechanisms (Trusolino L, 2002). As for colorectal cancers, MET expression levels as determined by reverse transcription-PCR, western blot, or IHC were found to correlate directly with cancer stage (Takeuchi H, 2003).
Recepteur d’Origine Nantais (RON) is a member of the MET receptor tyrosine kinase family (Ronsin et al, 1993). The ligand for RON was identified as macrophage-stimulating protein (MSP) (Rampino et al, 2002; Wang et al, 1994). Recepteur d’Origine Nantais induces cell transformation and epithelial tumorigenesis (Collesi et al, 1996; Santoro et al, 1996; Peace et al, 2001; Chen et al, 2002). In primary human cancer, highly expressed RON was observed in 59.2% (29 of 49 cases) of colorectal cancers and 47% (35 of 74 cases) of breast cancers and (Maggiora et al, 1998; Zhou et al, 2003).

Dimerisation by binding two monomers is the regulatory mechanism for the activation of tyrosine kinase receptors (Heldin, 1995). In some cases, formation of heterodimeric complexes allows interaction and cross-talk between different receptors of the same subfamily (Wada et al, 1990; Sliwkowski et al, 1994; Pinkas-Kramarski et al, 1996; Chow et al, 2001). Therefore, determining the clinical significance of the co-expression pattern of PTKs can provide important molecular targets for cancer therapy.

In the prior experiment of our groups (Lee et al, 2005; Cheng et al, 2005), co-expression of RON and MET suggests a shorter overall survival in patients with breast cancers or transitional cell carcinoma of the bladder. As crosstalk between RON and MET was observed in epithelial cancer (Chen et al, 1997; Maggiora et al, 2003), we investigated the clinical significance of RON and MET overexpression in human colorectal cancer.

2. Material and methods

(1) Patients and material

The records of 135 patients (74 men and 61 women) with colorectal cancer who underwent a primary resection of the tumor at the National Cheng Kung University Hospital between 1993 and 1998 were reviewed (Table 1).

Those patients who received adjuvant chemotherapy or radiotherapy before initial resection were excluded from this study. The observation time in this unselected cohort was the interval between diagnosis and last contact (death or last follow-up). Data were censored at the last follow-up for patients who had not relapsed and for those who had died. The mean duration of follow-up was 74.8 months. Histopathological and clinical findings were scored according to the tumor–node–metastasis (TNM) staging system of the Union Internationale contre le Cancer (Sobin 2002) (Table 1). The TNM stage and of the tumors were determined from the histopathological reports obtained at the time of resection.

(2) Immunohistochemistry

Tissue sections were obtained from a representative formalin-fixed paraffin-embedded tissue block of each patient’s tumor. Tissue blocks containing a transmural, full-thickness section of
adenocarcinoma, including the deepest pericolonic extension were selected from the slide review.

Immunohistochemical staining by the avidin-biotin-peroxidase complex method was done with an LSAB kit (DAKO). Briefly, 4-Am-thick sections were prepared and endogenous peroxidase activity was blocked with H2O2. Microwave antigen retrieval was done in 10 mmol/L citrate buffer (pH 6.0) at 750 W. The sections were incubated with primary antibodies at 4°C overnight. Antigens were detected using an LSAB kit (DAKO) and visualized using an aminoethyl carbazole substrate kit (AEC kit, Zymed Laboratories, San Francisco, CA). Finally, the sections were lightly counterstained with hematoxylin. Positive and negative controls were included in all runs. For negative controls, we omitted the primary antibodies. Positive controls consisted of colorectal cancer tissue known to express RON and MET.

(3) Evaluation of immunohistochemistry

All slides were interpreted by two independent observers blinded to the clinical outcomes. The expression of RON and MET were determined by staining intensity in the adenocarcinoma cells. Referring to the previous literature (Takeuchi H, 2003; Resnick MB, 2004), we used a four-tier score system in the following manner: negative 0, weak 1+, moderate 2+, or strong 3+. Besides, 0 and 1+ were defined as low expression; 2+ and 3+ were defined as high expression. For each case, the whole area of the slide was analyzed and a score was given by the strongest staining intensity.

(4) Statistical analysis ~

Kaplan–Meier curves were used to assess the immunohistochemical reactivity of RON and MET on overall and disease-free survival. The significance of various clinical characteristics was assessed by univariate analysis with the use of the log-rank test (Table 1). All analyses were performed with the use of SPSS statistical software.

3. Results

(1) Expression of RON & MET in primary colorectal adenocarcinomas ~

Immunohistochemical staining with an anti-c-Met Ab revealed positive brown signals in most (97.8%, 132/135) cases of colorectal cancers. 71.9% (97/135) showed moderate to strong staining intensity. Our observation that MET is strongly expressed by carcinomas confirms previous studies, documenting over-expression of MET in colorectal tumors. The MET protein expressed in cancer cells was distributed predominantly in the cytoplasm as a diffuse pattern. Membranous staining was also observed in the areas of increased intensity. The non-neoplastic mucosa near the cancer component was also stained with MET Ab. Most stromal cells were blue from the hematoxylin counter stain and as a result of negative c-Met staining. Negative control sections also did not stain.

Except for neoplastic and non-neoplastic epithelial parts, positive staining of endothelial cells in
tumor blood vessels and lymphocytes was also evident. The phenomenon was ever observed by Zeng Z. et al previously (Zeng Z, 2004)

Immunohistochemical staining with an anti-RON Ab revealed positive brown signals in most (95.6%, 129/135) cases of colorectal cancers. 73.3% (99/135) showed moderate to strong staining intensity. The staining pattern of RON is similar to that of MET, which is cell membranous and cytoplasmic. The positive staining of endothelial cells in tumor blood vessels and lymphocytes was also observed.

The staining intensity of RON or MET varied among cases and in different areas of the same tumor. Enhanced expression at the invasive front of cancer was observed. The phenomenon was ever observed by Pai R. et al previously.

(2) Survival among patients of different characteristics ~

The prognostic significance of the immunohistochemical expression of RON and MET was investigated by univariate analysis of data from the 135 patients with colorectal cancer. The staining intensity of RON or MET, TNM stage, MAC stage, and tumor grade significantly influenced 5-year disease-free and overall survival (P<0.1 for all comparisons) (Table 1 & 2). Furthermore, we combined the two biomarkers. The concurrent high expression of RON and MET conferred a worse prognosis than the expression of the single one (Table 2).

The highest 5-year disease-free survival was seen in patients with low RON/low MET tumors (88.9%), and patients with high RON/high MET tumors had the lowest 5-year disease-free survival (40.5%, P < 0.001). The 5-year disease-free survival in patients with high RON/low MET+ tumors and low RON/high MET tumors was 80.0% and 94.4%, respectively. A similar pattern was found for 5-year overall survival (Table 2).

Analysis of Kaplan-Meier recurrence curves (Fig. 1-4) revealed that increased staining intensity of RON or MET correlated with a shorter interval of disease free or overall survival. Furthermore, the concurrent highly expression of RON and MET conferred a worse prognosis than the high expression of the single one (Fig. 5 & 6).

4. Discussion

Our study suggests that high expression of either RON or MET correlates with short interval of disease free survival or overall survival. Concurrent high expression of RON and MET suggests a poorer prognosis.

Reviewing the literature, aberrant MET expression (usually overexpression) has been found in many kinds of solid tumors and correlates with poor prognosis. As for colon cancer, MET expression levels as determined by reverse transcription-PCR, western blot, or IHC were found to correlate directly with colon cancer stage (Takeuchi H, 2003). Our observation that MET is strongly
expressed by carcinomas confirms previous studies, documenting over-expression of MET in colorectal tumors (Wielenga VJM, 2000; Di Renzo MF, 1995; ). However, correlation of MET expression with recurrent disease or patient survival in colon cancer has rarely been reported.

In our study, the immunohistochemical expression level of MET correlates with short interval of disease free survival or overall survival. It supports the hypothesis that MET-related signaling events play an important role in the progression of colorectal cancer. However, a tissue microarray study of 134 patients reported by Murray B.R. revealed no significant association between MET expression by IHC and prognosis. It is difficult to explain the conflict between different laboratories. Tissue sampling (for microarray) or variations in tissue preparation and fixation may contribute to part of the conflict.

RON is overexpressed and constitutively active (by western blot and IHC) in some primary tumors and tumor cell lines. However, correlation of RON expression with recurrent disease or patient survival in colon cancer has not been reported. Our study is the first one to compare the RON expression with prognosis of patients with colorectal cancers. It indicates that high expression of RON correlates with a shorter interval of disease free survival and overall survival, which supports the hypothesis that RON-related signaling events play an important role in the progression of colorectal cancer.

Increasing data proves the existence of cross-talk between MET and different membrane receptors, suggesting a role in complex and interacting networks. The physiological meaning of these interactions and their consequences is not completely understood because adequate animal models to study them are unavailable. However, it has been convincingly shown that, in many cases, these interacting receptors can cooperate to promote tumorigenesis and/or metastasis.

In this study, high expression of both RON and MET is found in 59% of cases, and indicates the worst prognosis. This result is in agreement with those of the previous studies of bladder and breast cancers in our groups (Cheng HL, 2005; Lee WY, 2005). It supported that the formation of heterodimers of RON and MET receptors results in a more efficient signal (Santoro MM, 1996).

In our study, the staining pattern of MET was predominantly cytoplasmic and occasionally membranous, which was similar to that of RON. Increased staining intensity of MET or RON in cancer cells forming the invasive front were frequently observed, which is in agreement with a previous report of MET and EGFR (Resnick MB, 2004; Pai R, 2003). These results provide additional evidence that the deepest, most invasive adenocarcinoma may be the optimal region that reflects the most aggressive growth and metastatic growth potential of the adenocarcinoma. Besides, positive staining of RON and MET of endothelial cells in tumor blood vessels and lymphocytes was observed. The result is in agreement with a previous report of MET (Zeng Z, 2004), which supports that MET and RON may mediate tumor endothelial cell angiogenesis and contribute to the invasiveness of the colon cancer.

In summary, the results of our study indicate that RON-related molecular events are important in the progression of colorectal carcinogenesis. Evaluation of the expression pattern of MET and
RON is of great help in selecting colorectal cancer patients for more aggressive therapy.

References ~


