乳癌的 Kruppel-like factor 表現和臨床表現的關聯性

The Correlation of Expression of Kruppel-like factors (KLF) and the Clinical Manifestations of the Breast Cancer

中文摘要

乳腺癌是台灣女性因癌症致死的第四位，乳腺癌的發生率有逐年增加和年輕化的趨勢（40 歲[含]以下佔 29.3%）。近年來，台灣乳腺癌之年齡分佈的狀況和年輕女性所發生之乳腺癌臨床上較具有較侵犯性的表現與西方國家不同。此外，由於乳腺癌本身的異樣性特質，理想的乳腺癌治療方法不僅須依賴傳統的病理組織學、臨床表徵和常用之生物標誌（如 ER、PR、HER2/neu），還需探討與發現新的與預後相關的生物因子。由上所述，對台灣婦女乳腺癌的專門研究，成爲一個重要的問題。

Kruppel-like factors (KLFs)是一群鋅指樣轉錄因子（Zinc finger like transcription factors），最初被發現和細胞生長的調控有關，目前已知共有超過 20 種 KLFs。最近幾年有越來越多探討 KLF 在乳腺癌表現的研究，多數研究主要著重於 KLF 涉及的致癌機轉，而且有數篇研究認爲 KLF 爲乳腺癌新的預後因子。在乳腺癌最重要和最被廣泛探討的有 KLF4 和 KLF5，但研究主要局限於西方國家。爲了對本地乳腺癌提供更好的治療策略，本研究的目的是探討 KLF4 和 KLF5 在台灣婦女乳腺癌的表現情形和與乳腺癌的生物行爲相關性。

過去的文獻指出，KLF4 同時有抑癌基因和致癌基因的功能，KLF4 不僅促進癌細胞的增生，而且還會調節細胞外基質的產生。此外，較侵犯性的臨床行爲可能與 KLF4 在腫瘤細胞內的分布型態有關，在腫瘤細胞有明顯的核表現時，病人預後較差。KLF5 也同時具有抑癌基因和致癌基因的特性。KLF5 會促進癌細胞增生和轉化，而且具有高表現量的 KLF5 的乳腺癌病人的預後不良，這現象和 HER-2/neu 基因及 Ki-67 的表現量呈正相關性。高表現量的 KLF5 通常發生在 50 歲(含)以下的乳腺癌患者身上。

在本研究中，我們使用免疫組織化學方法探討 KLF4 和 KLF5 在非腫瘤及腫瘤（含浸潤癌或原位癌）乳腺組織的表現強度和表現型態。並同時統計 KLF4 和 KLF5 的表現與乳腺癌組織學特徵、臨床特徵和其他傳統的預後因子的相關性。

本研究共收集了 60 名乳腺癌患者，其平均年齡爲 47 歲，平均腫瘤大小爲 2.7 公分。臨床表現爲第一期的有 30%；第二期有 43.3%；第三期有 21.7%；第四期有 5%。經診斷後的平均追蹤時間爲 27 個月（範圍從 8 至 59 個月），其中只有一名患者是死於乳腺癌。本研究中 90%病例的乳腺癌的組織型態爲浸潤性管道癌（Invasive ductal carcinoma, IDC），且 66.7%爲中度分化。於病灶旁邊管道原位癌（Ductal carcinoma in situ, DCIS）有 60%的分化程度爲最差等級的。將 KLF4 和 KLF5 的免疫組織染色結果與這些病例的臨床表現作關聯性探討時，發現 KLF4 表現以細胞質和核爲主，其中 43.3%的病人爲腫瘤部分的表現強度比非腫瘤部分

強。若腫瘤細胞的 KLF4 核表現傾向≧25%的病例，有較高的癌症分期（p=0.006），並有較大的腫瘤（最大徑超過 2 公分，p=0.035）。KLF4 的表現也有年齡的相關性，即年齡超過 50 歲的病例，浸潤癌或原位癌的表現比非腫瘤部分來的更強（p= 0.007），而且，其浸潤性癌的分化也較差（p=0.033）。此外，我們還發現同一病例的浸潤性癌和原位癌之表現有一致性：若原位癌的表現越強，其浸潤性癌的表現也強（p=0.002）；核表現的傾向也有一致性（p<0.001）。KLF5 表現以細胞質爲主，其中 58.3%的病人爲腫瘤部分（含浸潤癌或原位癌）的表現強度比非腫瘤部分強。就 KLF5 方面，浸潤性癌若爲陰性或弱的細胞質染色時，比較強細胞質表現的乳癌有較好的組織學分化（p=0.035）。另外，同一腫瘤內 KLF5 的表現在浸潤性癌和原位癌有一致性：若原位癌的表現越強，其浸潤性癌的表現也強（p<0.001）；細胞質表現的傾向也具有一致性（p<0.001）。此外，KLF4 的表現強度和型態分別和以下因子的表現無相關：動情素接受器（p=0.271 和 p=0.925），黃體激素接受器（p=0.191 和 p=0.448），HER-2/neu（p=0.136 和 p=0.454），p53（p=1.000 和 p=0.925）和 p21 （p=0.572 和 p=0.367）。KLF5 的表現強度和型態分別和以下因子的表現無相關：動情素接受器（p=1.000 和 p=0.512），黃體激素接受器（p=1.000）和 HER-2/neu（p=0.520 和 p=0.443）。本研究發現 KLF4 表現與浸潤性癌的分期，腫瘤大小，病人年紀有正相關性，未能得出和已知文獻中提到 KLF4 核表現強，其臨床預後較差的結果；另一方面，KLF5 表現與浸潤性癌的分化程度有關，我們同時發現到 KLF5 的核表現主要是局限在非腫瘤性的乳腺組織（16.7%），且沒有任何核染色在原位癌和浸潤性癌發現，已發表的文獻並未提到此現象。雖然，我們目前還不知道這現象代表的生物意義，但這可能代表 KLF5 有抑癌基因的作用。我們觀察到 KLF4 和 KLF5 表現和乳腺癌的臨床表現有關，但其表現與否還無法作爲預測乳腺癌的預後和存活率，主要是由於本研究之病例追蹤時間不夠長，無法明確顯示 KLF4 與 KLF5 和存活率的相關性，所以精心設計的回顧性研究，配合上長時間的病人追蹤在研究 KLF4 和 KLF5 表現與乳腺癌預後及存活率的關聯性是必要的。

英文摘要

Breast cancer is the fourth cause of female cancer deaths in Taiwan with increased incidence and young age tendency (age?T40 years old, 29.3%). In recent years, the distinct age distribution and more aggressive clinical behavior in the young patient are noted in Taiwanese women and this phenomenon is different from that in the Western countries. Besides, due to the heterogeneity of breast cancer, designation of an ideal treatment protocol for breast cancer could not only be based on the traditionally histological, clinical, and biological markers (such as ER, PR and HER-2/neu) but also some new prognostic factors. Therefore, the specific study of breast cancer in Taiwan women becomes an important issue.

Kruppel-like factors (KLF) belong to a group of zinc finger like transcription factors

and are involved in regulating cell proliferation. KLFs have more than twenty subtypes. The studies of Kruppel-like factors in breast cancer are increased recently and are mainly focused on their roles in tumorigenesis. The KLFs are considered as new prognostic factors in breast cancers in some studies. Among them, KLF4 and KLF5 are most important and are broadly studied, but most studies are mainly in Western countries. In order to provide better treatment strategies for native breast cancers, the aim of this study is to evaluate the correlation of KLF4 and KLF5 expression with pathologic changes and clinical behaviors of breast cancers in Taiwanese women.

In the literatures, KLF4 has both tumor suppressor gene and oncogene functions. KLF4 can promote the proliferation of cancer cells and also can regulate production of extracellular matrix. More aggressive clinical manifestations may be associated with the cellular location of KLF4 in cancer cells. The patients have poor prognosis when nuclear localization of KLF4 in cancer cells. KLF5 also has both tumor suppressor gene and oncogene functions. KLF5 can facilitate the proliferation and transformation of cancer cells. Increased expression of KLF5 is a poor prognostic factor and is positively correlated with the expression of HER-2/neu and Ki-67 in breast cancer. KLF5 also has increased expression in breast cancer patients younger than 50 years old.

In this study, we used immunohistochemistry method to evaluate both staining intensity and staining pattern of expression of KLF4 and KLF5 in non-tumor and tumor parts (including invasive and in situ cancers) of breast tissues. We also analyzed the associations of expression status of KLF4 and KLF5 with histological features, clinical presentation and other prognostic factors of breast cancer.

We enrolled 60 breast cancer patients with the mean age 47 years old and the mean tumor size was 2.7 cm. The clinical presentation was stage I: 30.0%; stage II: 43.3%; stage III: 21.7%; and stage IV: 5.0%. The follow-up period of these patients ranged from 8 to 59 months (mean 27 months) and only one patient died of disease. Pathologically, most of them were invasive ductal carcinoma (IDC) (90.0%) and showed moderately differentiation (66.7%). The accompanied ductal carcinoma in situ (DCIS), if present, was predominantly highest grade (60.0%). The immunohistochemical study of KLF4 in cancer cells showed cytoplasmic and nuclear expression. The intensity of tumor part was stronger than non-tumor part in 43.3% patients. We evaluated the association of the immunohistochemical results of KLF4 and KLF5 and clinical manifestations of these patients. We found that more KLF4 nuclear expression in tumor cells positively correlated with more advanced stage ($p=0.006$) and larger size of the tumor (size more than 2 cm in maximal diameter, $p=0.035$). KLF4 expression was also age-related. KLF4 intensity was stronger in

tumor part than non-tumor part in patients older than 50 years old (p=0.007) and, in this setting, the invasive cancer tended to be poorly differentiated (p=0.033). Besides, consistent expression of KLF4 between DCIS and invasive cancers was also found: stronger intensity in DCIS accompanied with stronger intensity in invasive cancers (p=0.002), more predominant nuclear expression in DCIS with more predominant nuclear expression in invasive cancers (p<0.001). The expression of KLF5 in cancer cells was mainly cytoplasmic. The intensity of tumor part was stronger than non-tumor part in 58.3% patients. For KLF5, invasive breast cancers with negative or weak cytoplasmic expression showed better differentiation compared with strong cytoplasmic expression (p=0.035). Consistent expression of KLF5 between DCIS and invasive cancers was also found: stronger intensity in DCIS with stronger intensity in invasive cancers (p<0.001) and more predominant cytoplasmic expression in DCIS with more predominant cytoplasmic expression in invasive cancers (p<0.001). Moreover, there was no association between the following factors and the KLF4 expression intensity and pattern, respectively: ER (p=0.271 and p=0.925), PR (p=0.191 and p=0.448), HER-2/neu (p=0.136 and p=0.454), p53 (p=1.000 and p=0.925), and p21 (p=0.572 and p=0.367). There was also no correlation between the following factors and the KLF5 expression intensity and staining pattern, respectively: ER (p=1.000 and p=0.512), PR (p=1.000 and p=1.000), and HER-2/neu (p=0.520 and p=0.443).

Our study found that KLF4 expression is positive association with tumor stage, tumor size, and age but could not conduct the conclusion that nuclear KLF4 expression was an adverse prognostic factor proposed in the literatures. In the other hand, KLF5 expression was associated with the differentiation of invasive cancers. We also found that KLF5 nuclear localization was mainly restrictedly in non-tumor breast ducts and lobules (16.7%) and loss of nuclear expression in DCIS and invasive cancers, the finding not mentioned in literatures before. Although we didn’t study the biologic function of KLF5, it maybe presented a possible tumor suppressor gene-like function of KLF5. We found that there were associations of KLF4 and KLF5 expressions and clinical manifestations in breast cancers but the expressions of KLF4 and KLF5 were not enough to predict the prognosis and survival rate. The major cause was due to too short follow up period of our patients to exactly evaluate the association of survival rate and expressions of KLF4 and KLF5. Therefore, well-designed retrospective studies with adequate follow up period for studying correlation of expressions of KLF4 and KLF5 and prognosis and survival rate of breast cancers are necessary.