MicroRNA-485-5p targets keratin 17 to regulate oral cancer stemness and chemoresistance via the integrin/FAK/Src/ERK/ β-catenin pathway

Te-Hsuan Jang, Wei-Chieh Huang, Shiao-Lin Tung5,Sheng-Chieh Lin, Po-Ming Chen, Chun-Yu Cho, Ya-Yu Yang, Tzu-Chen Yen, Guo-Hsuen Lo, Shuang-En Chuang and Lu-Hai Wang*

Journal of Biomedical Science| Received: 18 January 2022| Accepted: 7 June 2022

Student : Pin-Yi Chen Advisor: Dr. Chien Kuo Tai Date:2022.10.07 Class room:R236

1. 簡述論文的概要以及重大發現

近年來頭頸癌成為世界上最致命的癌症之一，其中以口腔癌的比例最高，大約占60-70%，口腔癌的最主要的危險因子為檳榔、菸、酒。口腔癌早期發現早期治療，存活率高。目前口腔癌的患者多需要接受手術並術後接受放射線治療或化學治療就可以將腫瘤控制好。若是晚期口腔癌，則五年存活率只剩下 30%。晚期口腔癌的復發大都發生在3 年內，其中有 80%出現於局部或頸部，20%會發生轉移。Cisplatin/Carboplatin 是化學治療的藥物，但常因為抗藥性的產生而造成治療失敗與癌症復發。近年來有許多研究指出，癌症幹細胞是抗藥性、腫瘤復發和轉移的關鍵因素。因此作者決定探討癌症幹細胞和抗藥性的機制和找到藥物去有助於改善 OSCC 的治療·

在本篇研究中，作者利用一般和高侵襲性的口腔癌細胞找到 KRT17，也從病人檢體中發現晚期口腔癌患者的組織切片 KRT17 表現量高且存活率差·作者從 RT-qPCR 中發現當口腔癌幹細胞 KRT17 表現量高，且會去調控癌幹細胞與 EMT－也發現 KRT17會和 plectin 結合去調節 intergrin/FAK/Src/ERK/β-catenin 這條 pathway·根據文獻報導intergrin /Src/β-catenin 會去調節 CD44/EGFR，因此作者確認 KRT17 也會和 CD44/EGFR 呈現正相關·從 Targetscan 7.0 中找到 miR-485-5P 會去抑制 KRT17，也發現會間接去抑制幹細胞和抗藥性的特性·最後，作者將細胞株抑制 KRT17 並與 Cisplatin 一起使用時去打入小鼠中發現可以有效的去降低腫瘤的大小·

2. 對內容的疑問

作者在後續的實驗中找到 Src 的抑制劑 Dasatinib，但作者沒提到 Dasatinib 會對口腔癌細胞造成什麼影響，此藥物目前也只使用在白血病當中，且副作用也很大·但在本實驗中發現在口腔癌細胞中加入 Dasatinib 可以去降低 Cisplatin 和 Carboplatin 的抗藥性·

3. 論文的缺點與評論

此篇研究證實 miR-485 會調控 KRT17，也發現會影響 intergrin/FAK/Src/ERK/β-catenin 這條 pathway，而導致癌症幹細胞和抗藥性的產生·若此篇研究後續的動物實驗中若能使用 Cisplatin 加入 Dasatinib 的聯合治療，可以先觀察動物實驗結果如何，或許可用在高度表達 KRT17 的口腔癌病患中，讓病患存活率增加·

Jang et al. Journal of Biomedical Science (2022) 29:42
https://doi.org/10.1186/s12929-022-00824-z

Journal of Biomedical Science

RESEARCH Open Access

MicroRNA-485-5p targets keratin 17 to regulate oral cancer stemness and chemoresistance via the integrin/FAK/Src/ERK/β-catenin pathway

Te-Hsuan Jang[1,2,3†], Wei-Chieh Huang[3,4†], Shiao-Lin Tung[5,6], Sheng-Chieh Lin[3], Po-Ming Chen[3], Chun-Yu Cho[2], Ya-Yu Yang[2], Tzu-Chen Yen[7], Guo-Hsuen Lo[8], Shuang-En Chuang[2*] and Lu-Hai Wang[1,3,4*]

Abstract

Background: The development of drug resistance in oral squamous cell carcinoma (OSCC) that frequently leads to recurrence and metastasis after initial treatment remains an unresolved challenge. Presence of cancer stem cells (CSCs) has been increasingly reported to be a critical contributing factor in drug resistance, tumor recurrence and metastasis. Thus, unveiling of mechanisms regulating CSCs and potential targets for developing their inhibitors will be instrumental for improving OSCC therapy.

Methods: siRNA, shRNA and miRNA that specifically target keratin 17 (KRT17) were used for modulation of gene expression and functional analyses. Sphere-formation and invasion/migration assays were utilized to assess cancer cell stemness and epithelial mesenchymal transition (EMT) properties, respectively. Duolink proximity ligation assay (PLA) was used to examine molecular proximity between KRT17 and plectin, which is a large protein that binds cytoskeleton components. Cell proliferation assay was employed to evaluate growth rates and viability of oral cancer cells treated with cisplatin, carboplatin or dasatinib. Xenograft mouse tumor model was used to evaluate the effect of KRT17- knockdown in OSCC cells on tumor growth and drug sensitization.

Results: Significantly elevated expression of KRT17 in highly invasive OSCC cell lines and advanced tumor specimens were observed and high KRT17 expression was correlated with poor overall survival. KRT17 gene silencing in OSCC cells attenuated their stemness properties including markedly reduced sphere forming ability and expression of stemness and EMT markers. We identified a novel signaling cascade orchestrated by KRT17 where its association with plectin resulted in activation of integrin β4/α6, increased phosphorylation of FAK, Src and ERK, as well as stabilization and nuclear translocation of β-catenin. The activation of this signaling cascade was correlated with enhanced OSCC cancer stemness and elevated expression of CD44 and epidermal growth factor receptor (EGFR). We identified and demonstrated KRT17 to be a direct target of miRNA-485-5p. Ectopic expression of miRNA-485-5p inhibited

†Te-Hsuan Jang and Wei-Chieh Huang contributed equally to this work

*Correspondence: sechuang@nhri.edu.tw; luhaiwang@mail.cmu.edu.tw

[2] National Institute of Cancer Research, National Health Research Institutes, Miaoli, Taiwan
[3] Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan
Full list of author information is available at the end of the article