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ORIGINAL ARTICLE

Differential expressions of *MDM2* and *TAP73* in cancer and cancer-adjacent tissues in patients with non-small-cell lung carcinoma

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Abstract
Aim: To investigate the differences in mRNA and protein expressions of *MDM2* (mouse double minute 2 homolog) and *P73* in cancer and cancer-adjacent tissues in patients with non-small-cell lung carcinoma (NSCLC).
Materials and methods: We compared the protein expressions of MDM2 and P73 in lung cancer and cancer-adjacent tissues in NSCLC patients by IHC (immunohistochemistry) and WB (Western blot). We divided the NSCLC patients into two subgroups, adenocarcinoma and squamous carcinoma. The mRNA expressions of two main isoforms of *P73*, *TAP73* and *DNP73*, as well as the ratio of *DNP73*/*TAP73* were analyzed by qPCR (quantitative real-time PCR) in the two tissues in all NSCLC patients and in patients with adenocarcinoma or squamous carcinoma, respectively.
Results: WB results did not show significant differences in MDM2 and P73 protein expressions in lung cancer and cancer-adjacent tissues. However, IHC results indicated that MDM2 expression significantly increased in cancer tissues in female patients, but not male patients. In addition, *TAP73* mRNA expression significantly increased in cancer tissues in all NSCLC patients ($p = 0.002$) and in patients with adenocarcinoma ($p = 0.01$); while there was no significant difference in *DNP73* mRNA expression. Hence the fold-change of *DNP73*/*TAP73* ratio significantly decreased ($p = 0.0003$) in cancer tissues in all NSCLC patients and in patients with adenocarcinoma.

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Conclusions: *TAP73* mRNA expression significantly increased in cancer tissues than cancer-adjacent tissues in all NSCLC patients and in patients with adenocarcinoma. Meanwhile, the fold-change of *DNP73*/*TAP73* ratio was similar to *TAP73*. MDM2 protein expression significantly increased in cancer tissues in female NSCLC patients.

Introduction

Lung cancer is a malignant tumor with the highest morbidity and mortality, and is a serious threat to human health. The etiology of lung cancer is the interaction of environment factors (smoking,[1,2] air pollution, ionizing radiation,[3] and diet[4]) and genetic factors.[5] Lung cancer can be classified into two major types, SCLC (small cell lung cancer) and NSCLC (non-small cell lung cancer), according to the histopathology. The most common types of NSCLC are SC (squamous carcinoma), adenocarcinoma, and large cell carcinoma.[6] SC accounts for 50% of all lung cancer cases. SC is more common in elderly men and is correlated with smoking. SC is sensitive to CT (chemotherapy) and RT (radiotherapy) treatments. The best treatment for patients with SC is surgical approach in a combination of CT and RT.[7] The five-year survival rate of SC is relative high.[8] Adenocarcinoma is more frequently observed in female patients and is not correlated with smoking. The morbidity of adenocarcinoma has risen in recent years and it has become the main type of lung cancer in some countries. Although the therapeutic methods have been improved, the overall-survival rate of lung cancer has not improved in recent years.[9] Hence, a deeper understanding of the etiology of lung cancer is necessary for the development of new therapeutic approaches and the treatment of lung cancer.

TP53 is a classical tumor-suppressor gene[10] and is frequently altered in majority of the human cancers,[11] resulting in the expression of mutant P53 proteins with single-amino-acid substitutions within the DNA-binding domain (DBD).[12] Therefore, *TP53* plays an important role in maintaining the genome integrity.[13] *P73* and *P63* are two homologs of *TP53*. Unlike *TP53*, *P63* and *P73* regulate developmental processes rather than participate in the control of genome stability.[14] *P73* is located on human chromosome 1p36.3, and is consisted of 13 exons and 12 introns. It has been reported that P73 plays an important role in cancers.[15] P73 is involved in the control of programmed cell death,[16] and can be used as an indicator of cancer prognosis.[17] *P73* mutation is often resulted in a variety of tumors, including neurocytoma, CRC (colorectal cancer) and breast cancer.[18,19]

P73 encodes two isoforms, *TAP73* (transcriptionally active *P73*) and *DNP73* (dominant negative *P73*).[20] Studies show that *P73* mRNA expression is higher in cancer tissues than in healthy tissues, suggesting that *P73* might be a oncogene.[21] Evidence indicates that TAP73 can suppress tumors formation while DNP73 can promote tumor formation.[22] Studies have found that TAP73 and DNP73 are overexpressed in ovarian cancer, hepatocellular carcinoma and colon cancer, and their expression levels are correlated with the development and prognosis of cancers.[22–25] Accumulating evidence suggests that the overexpression of DNP73 transcript is associated with adverse prognosis and chemotherapy failure in several human tumors.[26] High DNP73/TAP73 ratio is associated with poor prognosis in acute promyelocytic leukemia (APL).[27] The expression of TAP73 and DNP73 can be elevated simultaneously in lung cancer. Hence, TAP73 and DNP73 interact with each other and play complex roles in regulating the proliferation and apoptosis of lung cancer.[28]

MDM2 is located on human chromosome 12q14.3-q15, and is one of the principal ubiquitin ligases that are responsible for P53 degradation.[29,30] MDM2 can regulate the activity, stability and function of P53[31] and can also interact with P73.[32,33] In MDM2-P53 system, P53 activation induces MDM2 transcription; while MDM2 activation inhibits P53 activity by binding to its activated area of transcription.[34] However, it is unclear whether MDM2 can regulate P73 activity.

Studies show that MDM2 and P73 can form heterodimers in vivo or in vitro. MDM2 does not promote P73 degradation,[35] but it can suppress P73 protein expression by binding to the N terminal of the p300/CBP; while P73 can stimulate the expression of endogenous MDM2. Hence, MDM2 is a negative feedback regulator of P73, and form a negative feedback loop with P73.[14] MDM2-P73 system plays an important role in the development of lung cancer.[36] It has been reported that MDM2 overexpression and P73 deficiency can induce genome instability and tumor development.[37,38]

To date, no study has reported the expressions of MDM2 and P73 in different types of lung cancers. Hence, in this study, we investigated the relationship between MDM2 and P73 in lung cancers, as well as the functions of TAP73 and DNP73 in the development and prognosis of lung cancer.

Materials and methods

Patients and materials

We calculated the estimated sample size based on our preliminary data. We selected 45 patients with lung cancer in our hospital from June 2016 to October 2016. The inclusion criteria included: (1) The patients had not received chemotherapy (CT), radiotherapy (RT), biological drug treatment (drugs that could bind to the specific cancer site and kill the cancer cells) and surgery; (2) the patients did not have other tumors (such as carcinoma); (3) the patients were suitable for surgery; (4) the patients did not have other non-cancer diseases according