

Original Article

High frequency of coexpression of maspin with p63 and p53 in squamous cell carcinoma but not in adenocarcinoma of the lung

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Received August 25, 2013; Accepted September 10, 2013; Epub October 15, 2013; Published November 1, 2013

Abstract: Maspin, a member of the serpin family of protease inhibitors, has been shown to inhibit tumor growth and suppress metastasis in several malignancies, including lung cancer. Previous studies have reported that p63 and p53 control maspin expression by transactivating the promoter. The present study analyzed immunohistochemical studies to determine the expression and coexpression patterns of maspin, p63 and p53 in non-small cell lung carcinoma, specifically squamous cell carcinoma and adenocarcinoma. The results showed that 83/86 cases (96.5%) of squamous cell carcinoma and 82/161 cases (50.9%) of adenocarcinoma included in this study were positive for maspin. There were 79/86 cases (91.9%) of squamous cell carcinoma and 16/161 cases (9.9%) of adenocarcinoma with positive expression for p63. In addition, 77/86 cases (89.5%) of squamous cell carcinoma and 99/161 cases (61.5%) of adenocarcinoma were positive for p53. Maspin, p63 and p53 expression were each significantly higher in squamous cell carcinoma than adenocarcinoma. Squamous cell carcinomas more highly co-express maspin and p63, as well as maspin and p53, when compared with adenocarcinomas. The high frequency of coexpression of maspin and p63, as well as maspin and p53, in squamous cell carcinoma, suggests that p63 and p53 may be involved in the pathway to control maspin expression. Therapeutic targeting on maspin, p63 and p53 molecules might be beneficial in the management of patients with squamous cell carcinomas of the lung in the future.

Keywords: Maspin, p63, p53, lung, adenocarcinoma, squamous cell carcinoma

Introduction

Maspin, a member of the serine protease inhibitor (serpin) family, was originally detected in normal breast epithelial cells [1]. Maspin expression was previously shown to be down-regulated as breast epithelial cells progressed to a higher grade of breast tumor and lost in metastasis [2]. In contrast, other studies found that maspin expression was upregulated in breast, ovarian, and pancreatic carcinoma cells and only minimally expressed in normal cells [3-6]. More recently, maspin expression and clinical significance have been described in non-small cell lung carcinoma [7-9].

Unlike most of the known protease inhibitors, maspin inhibits cell invasion by blocking cell motility and angiogenesis [10, 11]. While mas-

pin is known to function as a tumor suppressor, the underlying mechanisms of maspin at the molecular level are still unclear. Zou *et al.* suggested that p53 upregulates maspin expression by binding to the maspin promoter after observing a strong induction of maspin expression in breast and prostate cancer cells subsequent to p53 expression [12]. Transcription factors Ets, such as Pdef, and Ap1 have also been reported to transactivate maspin expression [13, 14]. Another study found that maspin expression is solely dependent on the presence of p63 [15].

p63, a homologue of p53, does not act like a classical tumor suppressor because it is rarely mutated or deleted in tumors. Instead, it regulates programmed cell death, development and differentiation [16]. The p63 gene encodes mul-

Coexpression of maspin with p63 and p53 in lung cancer

Table 1. Immunohistochemical expression and coexpression of maspin, p63 and p53 in squamous cell carcinoma and adenocarcinoma of the lung

	Squamous Cell Carcinoma		Adenocarcinoma	
n	86		161	
Markers				
maspin+	83*	(96.5%)	82	(50.9%)
p63+	79*	(91.9%)	16	(9.9%)
p53+	77*	(89.5%)	99	(61.5%)
maspin+/p63+	78*	(90.7%)	8	(5.0%)
maspin+/p53+	75*	(87.2%)	62	(38.5%)

*P<0.01 in comparison with adenocarcinoma of the lung. +, Positive staining.

multiple proteins with remarkably divergent abilities to transactivate p53 reporter genes and induce apoptosis [17]. It also has been reported that p63 acts as a substitute for p53 in transactivation of maspin [18], particularly in lung tumorigenesis [15]. While p63 expression was not seen in normal lung tissue, it has been observed in normal bronchial tissue with genomic amplification in the development of squamous cell carcinoma of the lung. Unlike squamous cell carcinoma, genomic amplification of p63 was not seen in adenocarcinoma [19].

The expression of maspin, p63 and p53 in non-small cell lung carcinoma has been evaluated separately. There is, however, limited information about the coexpression of maspin and p63 or p53 despite the potential role of p63 and p53 in transactivating maspin expression. The aims of this study are (1) to determine the expression frequency of maspin, p63 and p53 in non-small cell lung carcinoma, specifically squamous cell carcinoma and adenocarcinoma, and (2) to investigate the correlation between maspin and p63 expression, as well as between maspin and p53 expression, in squamous cell carcinoma and adenocarcinoma of the lung.

Method

Case selection

A total of 247 surgically resected squamous cell carcinoma and adenocarcinoma of the lung specimens were collected from the surgical pathology archives at University of Rochester Medical Center, Rochester, New York. These

included 161 cases of adenocarcinoma and 86 cases of squamous cell carcinoma.

Immunohistochemical analysis

Representative areas of squamous cell carcinoma and adenocarcinoma of the lung from the formalin-fixed, paraffin-embedded specimens were identified and tissue microarrays were constructed with two or more repeated tissue cores (1.0 mm in thickness) from individual cases. Sections of 4-µm in thickness were cut from the tissue microarrays and stained with hematoxylin and eosin to confirm the presence of the expected tissue histology within each tissue core. Additional sections from the tissue microarrays were cut for immunohistochemistry. Immunohistochemical studies were performed on the tissue microarrays. Tissue sections from the tissue microarrays were deparaffinized according to established procedures and rehydrated through graded alcohols. They were then washed in distilled water and placed in phosphate buffered saline. Antigen retrieval was performed by heating sections in a 99°C water bath with pH 6 Tris buffer or pH 9 EDTA buffer for 20 minutes followed by a brief cool down period. Slides were then rinsed with buffered saline for several minutes and placed on a Dako Autostainer. They were covered with fresh buffered saline to prevent drying during staining. All sections were incubated with various primary monoclonal mouse antibodies against maspin (1:500, clone G167-70, BD Biosciences, San Jose, CA), p53 (1:75, clone DO-7, DAKO, Carpinteria, CA) and p63 (1:50, clone BC4A4, Biocare, Concord, CA) at room temperature. The antibodies were detected using the Dako Flex HRP detection kit and counterstained with Dako Flex hematoxylin. A specimen with known maspin, p63 or p53 overexpression served as positive controls. Negative controls were performed by replacing the various antibodies with normal serum. Slides were mounted using a xylene based mounting medium and viewed under a light microscope.

Microscopic evaluation

Manual evaluation of maspin, p63 and p53 expression was performed by BC and HX blind-

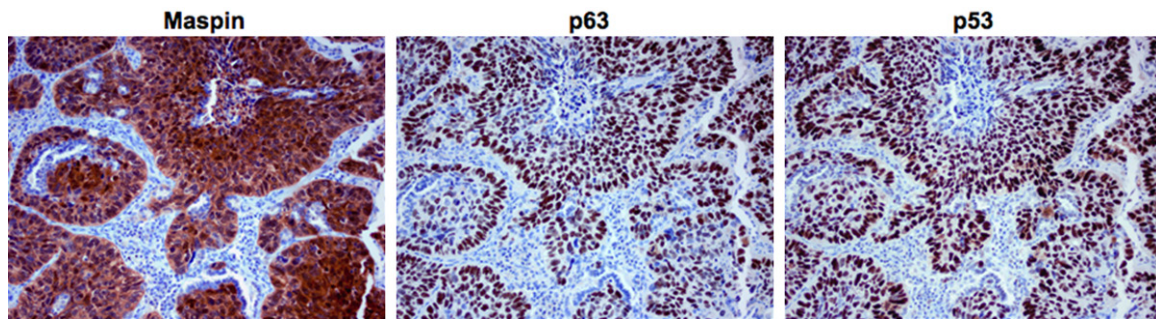


Figure 1. Maspin, p63 and p53 immunohistochemical expression patterns in squamous cell carcinoma of the lung. Squamous cell carcinoma was strongly and diffusely positive for maspin, p63 and p53 (original magnification x 200).

ed to all clinical and pathologic information. Discordant cases were reviewed by another pathologist and a final consensus was reached. Maspin, p63 and p53 immunoreactivities were semi-quantitatively assessed by the percentage of stained tumor cells and the intensity of staining. The percentage (0-100%) of positive tumor cells for maspin, p63 and p53 was determined. The intensities of maspin, p63 and p53 staining were graded as 0, 1+ (weak), 2+ (moderate), or 3+ (strong). Maspin, p63 and p53 proteins were considered expressed if more than 5% of tumor cells were stained and if there was at least a weak intensity of staining. Concordance was considered if both maspin and p63 or p53 were expressed in the same tumor.

Statistical analysis

Statistical Analysis was carried out by the SAS system. The Fisher's exact test and χ^2 -test were used to compare positivity rate of maspin, p63 and p53 between squamous cell carcinoma and adenocarcinoma of the lung. These tests were also used to assess the concordance of maspin and p63 or p53 positivity in the two types of non-small cell lung carcinoma. A *p*-value of less than 0.05 was considered statistically significant.

Results

Both nuclear and cytoplasmic maspin expression was observed in squamous cell carcinoma and adenocarcinoma of the lung. p63 and p53, however, had only nuclear staining. The expression of maspin, p63 and p53 were distributed throughout the full thickness of the lesion in squamous cell carcinoma and adenocarcinoma.

Table 1 summarizes the immunohistochemical staining results. Maspin, p63 and p53 were expressed in squamous cell carcinoma of the lung with the majority of cases exhibiting strong and diffuse positivity. The frequencies of their staining were 83/86 (96.5%) for maspin, 79/86 (91.9%) for p63, and 77/86 (89.5%) for p53. There were similar diffuse patterns of staining between maspin, p63 and p53 in squamous cell carcinoma (**Figure 1**). When the concordance between maspin and p63 expression was compared, the frequency was 78/86 (90.7%; **Table 1**). When the concordance between maspin and p53 was compared, the frequency was 75/86 (87.2%; **Table 1**).

The immunohistochemical staining results of the lung adenocarcinoma were more variable. Adenocarcinoma had a much lower positivity for all of the markers compared with squamous cell carcinoma. The staining frequencies of maspin, p63 and p53 were 82/161 (50.9%), 16/161 (9.9%), and 99/161 (61.5%), respectively (**Table 1**). The patterns of staining were similar between maspin, p63 and p53 when positive. However, the markers were not consistently coexpressed in the same tumor (**Figure 2**). The frequency of concordance between maspin and p63 was 8/161 (5.0%; **Table 1**) and between maspin and p53 was 62/161 (38.5%; **Table 1**).

Statistical analyses of the immunohistochemical staining results revealed that the positivity of maspin in squamous cell carcinoma was significantly greater than adenocarcinoma ($p < 0.01$). The differences between squamous cell carcinoma and adenocarcinoma were also statistically significant with $p < 0.01$ for both p63 and p53 expression. When concordance

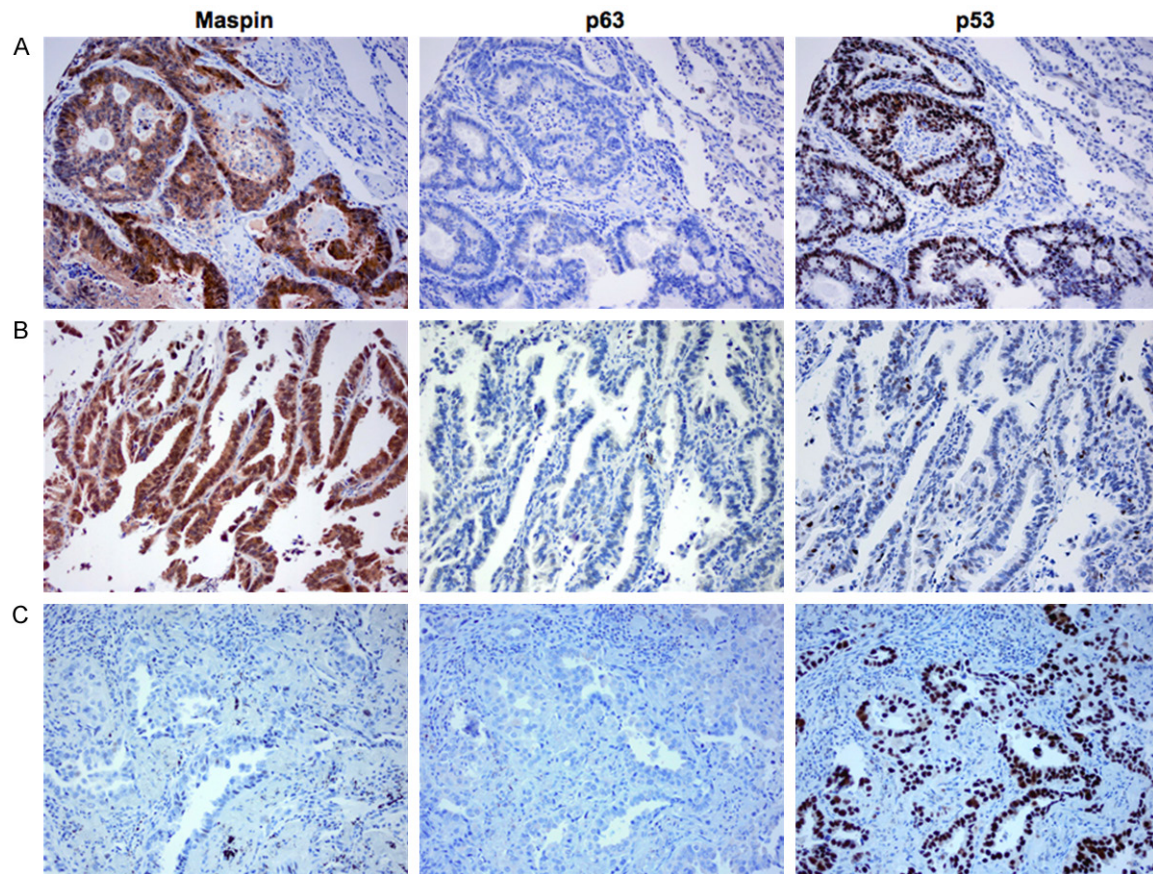


Figure 2. Maspin, p63 and p53 immunohistochemical expression patterns in adenocarcinoma of the lung. A: Adenocarcinoma co-expressing both maspin and p53, and exhibiting p63 negativity. B: Adenocarcinoma exhibiting maspin positivity, and both p63 and p53 negativity. C: Adenocarcinoma exhibiting both maspin and p63 negativity, and p53 positivity (original magnification x 200).

rates were evaluated, the coexpression rate of maspin and p63 in squamous cell carcinoma was significantly higher than the rate in adenocarcinoma ($p < 0.01$). There was also significant difference of $p < 0.01$ between the coexpression rates of maspin and p53 in squamous cell carcinoma and adenocarcinoma.

Discussion

In this study, we reported significantly higher expression of maspin, p63 and p53 in squamous cell carcinoma of the lung when compared with adenocarcinoma. The results extended beyond previous observations of individual expression by examining the coexpression of maspin and p63, as well as of maspin and p53. Squamous cell carcinoma was more likely to coexpress maspin and p63 when compared with adenocarcinoma. Squamous cell carcinoma was also more likely to coexpress

maspin and p53 when compared with adenocarcinoma.

Maspin, a member of the serpin family of protease inhibitors, was first shown to inhibit tumor growth and suppress metastasis in breast cancer [1]. To date, several studies have investigated the role of maspin in lung tumorigenesis. These studies have generally focused on comparing the levels of maspin expression in normal lung tissues and various lung carcinomas. They also examined the relationship between maspin expression and prognosis. Normal epithelial cells in the proximal airway were found to express maspin [20]. In lung carcinomas, some studies reported a significant increase in maspin expression in squamous cell carcinoma that was higher than in other histological types, such as adenocarcinoma. These studies also found maspin as an independent factor in predicting a favorable prognosis in patients with

squamous cell carcinoma of the lung [7-9]. In fact, Akyildiz *et al.* found the sensitivity and specificity of maspin positivity to detect adenocarcinoma of the lung to be only 59% and 73%, respectively [21]. However, others found maspin expression to have favorable prognostic significance in adenocarcinoma [22-24]. Yet another study found that overexpression of maspin in both adenocarcinoma and squamous cell carcinoma is an unfavorable prognostic factor [25]. The results from this study were consistent with previous studies that found maspin expression in squamous cell carcinoma to be significantly higher than adenocarcinoma. This suggests that maspin plays a more functional role in the tumorigenesis of squamous cell carcinoma than in adenocarcinoma.

The precise mechanisms of maspin as a tumor suppressor gene are still unclear. Different pathways have been suggested for maspin expression. Both p53 and p63, a homolog of p53, are potential regulators of the maspin gene. One of the earlier studies found p53 binding to the maspin gene promoter and upregulating the maspin expression [12]. Other studies reported both p63 and p53 transactivate the maspin promoter [15, 18]. In the present study, the high concordance rate of maspin and p63, as well as of maspin and p53, in squamous cell carcinoma of the lung supported the potential role of p63 and p53 as transactivators of maspin expression. However, maspin may not be involved in the regulation of p63 and p53 expression in adenocarcinoma.

In conclusion, maspin, p63 and p53 are highly expressed in squamous cell carcinoma of the lung and expression concordance between maspin and p63 or p53 is much higher in squamous cell carcinoma, but not in adenocarcinoma. These findings indicate that both p63 and p53 may control maspin expression in squamous cell carcinoma more likely than in adenocarcinoma of the lung.

Disclosure of conflict of interest

None.

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