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Differentiated Expression of Membrane Type Metalloproteinase (MMP-14, MMP-15) and Pro-MMP2 in Laryngeal Squamous Cell Carcinoma. A Novel Mechanism: Commentary on Research Study

Bodnar Magdalena[1*] **and Marszałek Andrzej**[1,2]

[1]*Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland*

[2]*Chair of Oncologic Pathology and Prophylaxis Poznan University of Medical Sciences & Greater Poland Cancer Center Poznan, Poland*

[*]**Corresponding author:** Bodnar Magdalena, Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland, Tel: 48 52 585 42 00; E-mail: magdabodnar@o2.pl

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Introduction

Laryngeal squamous cell carcinoma (LSCC) is the most frequent tumor of head and neck area, characterized by an aggressive biology, rapid progression, but in particular the tendency to metastases. The successful treatment remains an important therapeutic issue [1]. The regulation of matrix metalloproteinases (MMPs) signalling pathway axis (MMPs/MT-MMPs/TIMPs) (matrix metalloproteinases; membrane type metalloproteinases; tissue inhibitors of matrix metalloproteinases) play a crucial role in cancer transformation and progression [2]. The alternation in MMPs signalling pathways (the canonical and noncanonical pathways) underlies the different MMP/MT-MMP/TIMP expression both in cancer cells, and in tumor microenvironment [3,4]. The multiple interactions between matrix metalloproteinases activate tumor cells to remodel their microenvironment, enhances the aggressive potential of cancer cells, and in consequence leads to metastatic potential [4,5]. The evaluation of microenvironmental parameters, and their interactions with tumor cells, may provide important information about tumor biology, and propose possible new therapeutic strategies.

Aims and Methods

The aim of study was to evaluate the pro-MMP2, MMP14 and MMP15 localization, and their expression levels. Additionally and correlation between analyzed antigens in tumor cells and tumor stroma in primary laryngeal squamous cell carcinoma was considered.

The studies were performed on formalin fixed paraffin embedded LSCC tissue sections, and the control tissue sections, which contained a disease free normal mucosa, taken at least 2 cm away from the tumor resection margins. The analyzed tissue material in taking cases was divided according to lymph node involvement in given patients. The immunohistochemical studies were performed using primary antibodies against: pro-MMP2, MMP-14, MMP-15. The protein expression was evaluated according to modified Remmele-Stegner scale (0-15), as the ratio of the protein expression intensity and the number of positively stained tissue area. The statistical analysis was performed using SPSS 14.0 (Statistical Package for Social Sciences 14.0), using nonparametric tests: U Mann-Whitney, Kruskal-Wallis, Wilcoxon, and the classification tree test module. The differences were considered to be statistically significant when $p < 0.05$.

The Study Findings

The expression of pro-MMP2 was found in 58% primary tumor cases of SCC, MMP-14 in 78% primary tumor cases of SCC, MMP-15 in 98% primary tumor cases of LSCC. Higher expression of pro-MMP2 was seen in the tumor cells, compared with the surrounding stroma. Thus, MMP-14 and MMP-15 protein expression was higher in the tumor stroma compared to tumor cells. A statistically significant difference between the MMP-14 and MMP-15 expression in the tumor cells and surrounding stroma ($p<0.05$) was found.

Statistical analyzes performed according to the lymph node involvement, revealed decreased expression of pro-MMP2 in cancer cells, and in the tumor stroma in patients with lymph node metastases vs. cases without lymph node involvement. Also decreased expression of MMP-14 was found in the tumor cells and stroma, in patients with metastases to the lymph nodes. In the studied groups we observed statistically significant differences in the expression of pro-MMP2, MMP-14 in N(0) *vs.* N(+) group ($p<0.05$).

The analysis of the classification trees module revealed, that decrease expression of MMP-14 (IRS ≤ 1) and high levels of pro-MMP2 expression (IRS>3) are more probable with lymph node metastases (Figure 1A). Moreover, increased risk of metastasis was related to high MMP-15 protein expression (IRS>2) with simultaneous decrease expression of MMP-14 (IRS ≤ 1) (Figure 1B).

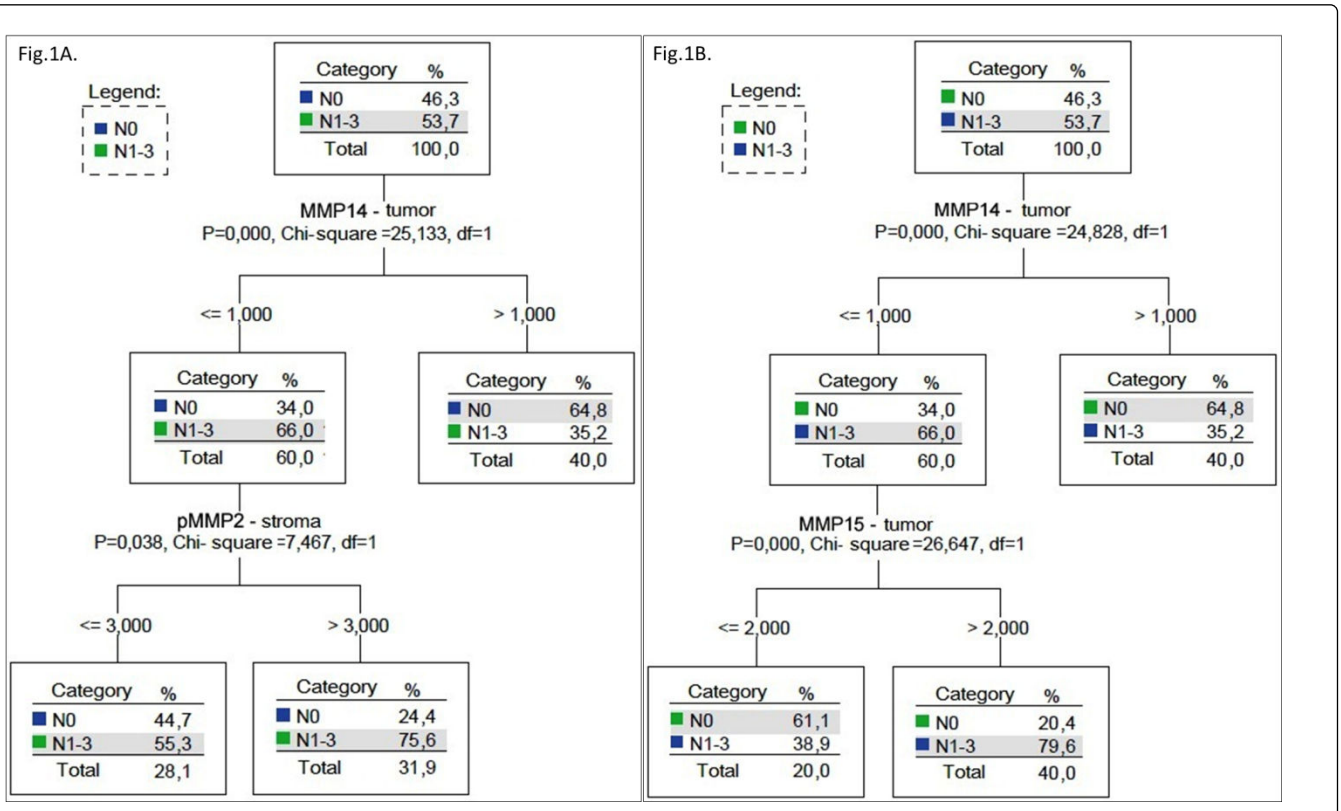
Discussion

Alternations and multilateral signalling pathways within cancer cells are the main target in cancer biology studies [6]. Signals from tumor microenvironment play a pivotal role in the tumor transformation, progression and metastasis [7]. Therefore, new findings highlighted the therapeutic strategies focused on the tumor stromal cells, and the mechanisms involved in the microenvironmental interactions with tumor cells.

Matrix metalloproteinases are multi-domain zinc-dependent proteolytic enzymes, involved in the extracellular matrix (ECM) remodeling [8]. During cancerogenesis increased synthesis of MMPs both in tumor cells, and tumor stroma, was sighted to increases the degradative processes, which may result in increased tumor cell migration [8].

Matrix metalloproteinases are produced and secreted as inactive proenzymes. Their activation occurs through the removal of prodomen of inactive MMPs molecule, which leads to the unveiling of the active site of the specific enzyme [9]. Under physiological conditions, their activity is regulated by endogenous tissue inhibitors of metalloproteinases, and membrane type of matrix metalloproteinases. TIMPs are able to bind to the C-terminal fragment of specific MMPs within the incovalent bindings, and in consequence inhibit the MMPs

activity [10]. However, TIMPs are involved in the degradation, but also in synthesis of extracellular matrix components by regulating both processes, and thus stimulate cancer progression [10]. Membrane type matrix metalloproteinases, especially MMP14 (MT1-MMP) and MMP-15 (MT2-MMP), have been described as direct-acting, "pro" invasive enzymes, activators of pro-MMPs, which are directly involved in tumor progression [11].



Figures 1: 1A) Classification tree of expression of matrix metalloproteinases (MMP-14) and pro-MMP2; 1B) MMP-14 and MMP-15.

MMP-2 is a major matrix metalloproteinase which plays a key role in the degradation of the basement membrane and is associated with accelerated tumor progression [12]. The activation of MMP-2 occurs through several signalling pathways. One of the main MMP-2 activation pathways involves activation by transmembrane metalloproteinases on the cell surface in two different mechanisms [3,13]. First activation pathway composes of several steps and requires the presence of tissue inhibitor of metalloproteinase-2 (TIMP-2), and MT1-MMP [3]. It can be divided into two activation steps. At the beginning MMP-14/TIMP-2 dimer is being formed, which inhibits MMP-14 activity, and allow TIMP-2 to anchor the pro-MMP2 (by binding with pro-MMP2 haemopexin domain). Subsequently, pro-MMP2 can be cleaved by an adjacent MMP-14 molecule, and finally another MMP-2 removes the remaining prodomain which, in consequence, leads to metalloproteinase activation [3]. According to second mechanism, TIMP-2/pro-MMP2 complex is formed and subsequently MMP-14 binds to this complex, for further dimerization [3]. Recent studies have revealed an alternative activation pathway for MMP-2 by membrane type-2 metalloproteinase (MT2-MMP, MMP-15). It has been found, that MMP-15 plays a similar role to MMP-14 in tumor progression [14]. Research presented in our finding demonstrated a statistically significant difference between the MMP-14, MMP-15 and pro-MMP2 expression, and the presence of lymph node metastases. In our studies, expression of MT-MMPs was found not only on cancer cells but especially on the tumor stroma cells. We have found decreased expression of MMP-14 in patients with lymph node involvement. Moreover, we have found, that risk of metastasis is related to high MMP-15 protein expression (according to analyzes of classification trees module). These results might suggest that in some cases, cancer cells could still demonstrate the ability to invade the stroma and to form metastases, without expression of MMP-14. This may also suggest, that there is an alternative mechanism for tumor progression, which is stimulated by MMP-15, but through a pathway independent to those which require MMP-14 involvement.

Analysis of classification trees in our studies also showed that patients with decreased expression of MMP-14 and pro-MMP2 had no metastasis to regional lymph nodes. Also, decreased expression of MMP-14 with simultaneously increased expression of pro-MMP2, predisposes to lymph node metastases. In our studies, metastases were found in approximately 76% of cases, with increased expression of pro-MMP2 and simultaneously decreased expression of MMP-14. Moreover, analysis of MMP-2-MMP14 ratios performed in our study revealed statistically significant differences in MMP2-MMP-14 ratios (p=0.000001) N(0) *vs.* N(+). These results support the conclusion about the alternative route for the activation of metalloproteinase-2.

Furthermore, recent findings highlighted the role of the specific EMMPRIN (extracellular matrix metalloproteinase inducer, CD147) factor which is located on the surface of cancer cells [15,16]. It was shown that EMMPRIN stimulates the synthesis of several MMPs (e.g.

MMP-2) by fibroblasts and endothelial cells [15,16]. EMMPRIN forms the border between tumor infiltration and stroma, increases the production of pro-MMP-2 activators, such as MMP-14 and MMP-15 [15,16].

Conclusion

Studies on the interactions between different types of metalloproteinases and their signalling/activating pathways, especially those involved in the degradation of basal membrane components (MMP-2, MMP-9), their inhibitors (TIMP-1, TIMP-2, TIMP-3) and transmembrane molecules (MMP-14, MMP-15), will improve the understanding of the tumor invasion and metastatic process. Nevertheless, the use of these factors, as prognostic indicator of clinical course in laryngeal squamous cell carcinoma, should be always supported by clinical informations', including primary tumor growth, the presence of lymph node and distant metastases, as well as the information about HPV infection.

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