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Short Communicaiton

Nuclear maspin expression: A biomarker for budding assessment in colorectal cancer specimens

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ABSTRACT

Aim: To evaluate the maspin expression in colorectal carcinomas (CRC) and its possible role in quantification of the tumor budding.

Methods: The tumor budding was prospectively quantified in 49 consecutive cases of patients that underwent surgical resection for CRC. The cases were divided in two groups: group A (n = 23) − low budding (< 5 tumor buds per high microscopic field) and group B (n = 26) − high budding CRCs (≥ 5 buds). Maspin expression was evaluated in the tumor core and the buds from the hot spot area in 44 of the microsatellite stable adenocarcinomas. Its expression was quantified as negative, cytoplasmic only, nuclear only or mixed expression (cytoplasm and nucleus).

Results: Compared with group A, a higher pT ($p < 0.0001$) and pN stage ($p = 0.0001$) and infiltrating aspect at macroscopic evaluation ($p = 0.0081$) was identified in group B. No correlation between the maspin expression in the tumore core and the budding grade was noted ($p = 0.14$). Compared with the tumor core, the cytoplasm to nuclear translocation of maspin was more frequently observed in cases from group B than A (n = 0.0063).

Conclusion: For the colorectal carcinomas, the infiltrative aspect at macroscopic evaluation and nuclear maspin in the buds might be used as indicators of risk for lymph node metastases. Maspin nuclear expression in the buds may be helpful for a proper budding assessment and may serve as a negative prognostic factor.

1. Introduction

For patients with colorectal cancer (CRC), the newest international guidelines indicate using of the tumor budding degree as an independent prognostic factor [1–4]. As definition, tumor buds represent single cancer cells or small clusters composed of no more than five tumor cells detached in the stroma at the invasive tumor front level [2]. They can be quantified on the conventionally slides stained with Hematoxylin and Eosin (HE) but using keratin is also indicated to identify the hot-spot areas [1,4]. Due to lack of reproducibility and difficulty of quantification and grading, this prognostic parameter was not included yet in the TNM staging system of CRC [3].

In this paper we present our preliminary data regarding tumor budding quantification and the correlation of tumor budding degree with clinicopathologic parameters in a prospective cohort of patients with CRC. The tumor budding was quantified on HE stained slides based on the recently published consensus [4]. As an original aspect, we have also examined the subcellular expression of Maspin, known to be a prognostic factor in CRC, in tumor core versus buds.

Maspin is a serine protease that is thought to inhibit tumor cell proliferation and angiogenesis and promotes apoptosis. It can be immunohistochemically (IHC) expressed in the tumor cells cytoplasm but can also show nuclear and mixed (cytoplasm and nucleus) expression [5–7]. In CRCs, we previously proved that subcellular expression of Maspin may be used as a prognostic parameter. The cytoplasmic expression seems to indicate the best prognosis, nuclear staining represents an indicator of aggressiveness and decreased survival time, while negative or mixed staining determines an intermediary outcome [5–7]. In this paper the significance of maspin subcellular localization in the CRC buds was explored.

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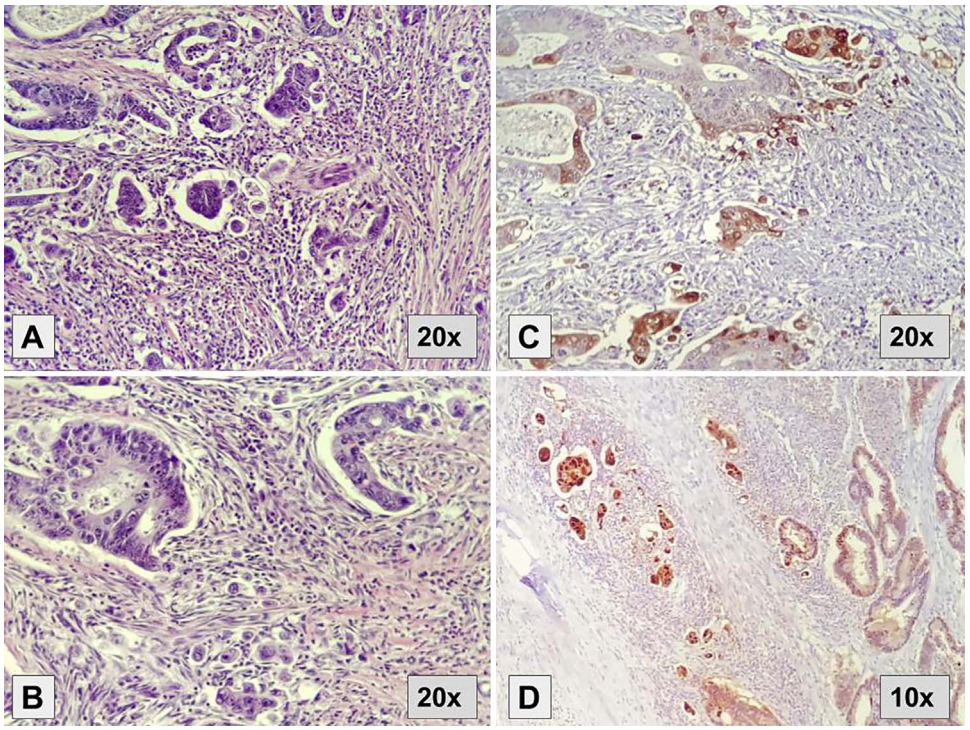

Fig. 1. Colorectal carcinomas with inflammatory stroma, explored with Haematoxylin & Eosin (A, B) and Maspin staining (C, D). Cytoplasm to nuclear translocation of maspin can be seen in the tumor buds (C,D).

2. Material and methods

In this study we have included 49 consecutive patients with CRCs. In all of the cases the surgical colorectal specimens were evaluated for diagnostic purposes. The 7th edition of the AJCC was used for tumor staging. Radiotherapy was not performed in any of the cases. All of the patients survived for more than 4 months after diagnosis. Cases with and without lymph node metastases were included, without cases with systemic metastases. All of the cases were microsatellite stable (MSS) adenocarcinomas.

The tumor buds were quantified under light microscopy on histology slides stained with HE. We have used the criteria proposed by Ueno H et al. in 2012 [1] adapted upon the International Tumor Budding Consensus Conference (ITBCC) criteria adopted in 2016 [4]. After careful examination of the invasion front and identification of the hotspot areas in low-power view, single tumor cells and clusters of tumor cells were counted with a 20 x objective lens (0.785 mm^2 field area). The cases were divided in two groups: group A (n = 23; 46.94%) – low budding cases (< 5 tumor buds) and group B (n = 26; 53.06%) – high budding group (≥ 5 buds, G2 and G3, 53.06% of cases) (Fig. 1).

In 44 of the cases (22 from group A and 22 from group B), the tumor buds were also counted using the IHC marker Maspin (clone EAW24, dilution 1:50, incubation in 0.01 M citrate buffer, pH 6.0, Novocastra, Newcastle Upon Tyne, UK). Based on the criteria previously presented in literature [6,7], taking into account the Maspin expression, the cases were classified as showing no expression, cytoplasm only positivity, nuclear only positivity and mixed expression (nucleus and cytoplasm). Maspin expression was evaluated in the tumor core and tumor buds (Fig. 1).

Statistical analysis was performed with GraphPad InStat 3 software. A p-value < 0.05 (95% confidence interval) was considered statistically significant, calculated with Fisher's exact test and Yate's continuity correction.

3. Results

3.1. Quantification on tumor buddings using HE stained slides

This study comprised 35 males and 14 females (M:F ratio = 2.5:1) with a median age of 65.96 ± 12.16 years (range from 33 to 83 years). Tumor budding degree was not correlated with gender, age, tumor size or distance up to the circumferential margin. A slightly predominance of poorly differentiated adenocarcinomas was noted for group B, compared with group A (Table 1).

Compared with group A, the cases from group B were mostly located on the rectum and shown an infiltrating aspect at macroscopic examination. All of the four cases diagnosed in pT1 and pT2 stages belonged to the group A. The pT4 cases predominantly showed a higher budding degree while the pT3 ones were rather included in the group A (p = 0.008). Statistically significant correlation was also noted between the grade of tumor budding and the number of lymph node metastases (p = 0.0001). All of the cases diagnosed as pN2 were included in the group B (Table 1).

3.2. Maspin expression in tumor core cells versus tumor buds

In the tumor core, Maspin positivity was noted in 34/44 cases (77.27%). The cytoplasmic positivity predominated in both group A and B (p = 0.14). No aberrant Maspin expression was seen in the inflammatory or stromal cells (Fig. 1). No differences between the budding grade in the intra- versus peritumoral areas were observed.

In the budding areas, a significant cytoplasm to nucleus translocation was observed in both groups. The nuclear expression only was more significant in the group B compared with group A (p = 0.02). In group A the cytoplasm expression in core was rather transformed in mixed positivity (Table 2).

4. Discussion

The present study confirms the association of tumor budding grade with pT and pN stages [2,4], highlighting the prognostic potential of this parameter. It was even postulated that the high tumor budding