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# Expression of maspin in testis tumors with germ cells and its relation with angiogenesis factors

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**Background/aim:** We aimed to evaluate the importance of maspin expression in testicular tumors with germ cells, its effect on prognosis, and the relation with angiogenesis factors.

**Materials and methods:** The paraffin blocks of the orchiectomy materials of 32 patients who had undergone orchiectomy due to testicular tumors were taken within the scope of the study. The specimens of the cases included in the study group were reexamined under light microscope.

**Results:** While just one maspin-positive sample was found in the seminoma cases, maspin stained positively in 6 of the nonseminoma germ cell tumors (NSGCTs). No statistical difference was found between maspin and tumor stage, size, alpha fetoprotein values, vascular endothelial growth factor, Ki-67, and CD31. A statistically positive correlation was only determined between maspin and p53 ($P < 0.001$).

**Conclusion:** Maspin protein, whose expression in some tumors is accepted as a poor prognostic factor, is also expressed in testicular tumors with germ cells. However, according to our study, it is difficult to say whether this protein is a favorable or poor prognostic factor in testicular tumors and to understand how the effect mechanism works. The positive correlation between maspin and p53 in the NSGCTs makes us think that maspin might have displayed an effect on the p53 pathway.

**Key words:** Testicular tumor, germ cell, maspin, vascular endothelial growth factor, p53

## 1. Introduction

Maspin (mammary serine protease inhibitor, serpin B5) is a serine protease inhibitor in the serine superfamily (1). Serine protease inhibitors are a large protein family related to inflammation, apoptosis, angiogenesis, and embryogenesis (2). Maspin is an intracellular protein that can dissolve in cytoplasm and can be found in different locations in the cell (3). The varied locations are important because abnormal localization in some tissues indicates neoplasia. For instance, whereas maspin in cytoplasm is associated with a poor prognosis, maspin in the nucleus is associated with benign lesions (2,3).

Maspin has been studied in many organs in the body (1–4). Most studies are related to prostate cancer in urology. A high rate of maspin release was shown in normal prostate epithelium cells. However, maspin release decreased in cells with prostate cancer and disappeared in metastases (4,5). Researchers have suggested that maspin positivity in prostate and lung cancer had an apoptosis-like effect (6). In a study carried out on renal tumors, cytoplasmic staining of maspin in kidney tumors was shown, and in renal cell carcinomas, the decrease in maspin expression was correlated with tumor growth and advanced pathologic stage. Therefore, the decrease in maspin expression was thought to be associated with a poor prognosis (7).

No detailed study related to maspin and the testes (normal testis tissue or tumors) has been published in the literature. Only two studies gave brief information about the fact that there is maspin expression in testis tissue (8,9). No studies have shown a relation between maspin and testicular tumors. In this study, the presence of maspin expression in the testis and the effects of maspin protein on tumor progression were evaluated, and its relation with angiogenesis factors such as vascular endothelial growth factor (VEGF), p53, Ki-67, and thrombocyte endothelium adhesion molecule-1 (PCAM-1 or CD31) was examined.

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## 2. Materials and methods

Paraffin blocks in the archives of the Başkent University Faculty of Medicine's Department of Pathology, comprising orchiectomy material from 32 patients diagnosed with a germ cell tumor (GCT) after they had undergone radical (inguinal) orchiectomy due to testis tumors, were used in this study. The study was approved by the Başkent University ethics committee. Sections from the paraffin blocks of 5 µm that included the entire tumor (seminomatous and nonseminomatous tumor areas were separated in mixed germ cell tumors) were prepared, and the sections were put on polylysine slides. CD-31 (Biocare, REF-CM347A, LOT101209-R1, 1/300 dilution), Ki-67 (Spring, Clone D0-7, RTU), and maspin (Lifespan, LOT# 18780, 1/50 dilution) primary antibodies were applied with a standard immunohistochemical staining procedure. The specimens were then screened under a light microscope. Brown-orange staining was accepted as positive owing to the chromogen used.

The mean age of the 32 GCT patients was 30.3 years (between 19 and 63). After the patient files were reexamined and the age and preoperative human chorionic gonadotropin (hCG) and alpha fetoprotein (AFP) values were obtained from the pathology reports and samples stained with hematoxylin and eosin, data on the histological type, tumoral mass size, tumor stage, lymphovascular invasion, rete testis stiffness, and lymph node stiffness were collected. The comparisons and statistical analyses were performed using the prognostic factors in the European Association of Urology's (EAU) testicular tumor guidelines (10).

### 2.1. Histopathologic evaluation

The positive staining pattern of the maspin protein was examined in the nucleus and cytoplasm of the tumor cells: cytoplasmic staining for VEGF and nuclear staining for p53. No nuclear staining was detected with maspin in any material. Positivity was assessed as 0 (1%–5% cells positive), + (6%–50% cells positive), and ++ (51%–100% cells positive). The positively stained cell count rate was calculated in approximately 1000 tumor cells in five large magnification areas for Ki-67 (5-HPF, 400× magnification). CD-31 was calculated by eliciting the average number of veins in five large magnification areas.

### 2.2. Statistical analyses

In the statistical assessment of the results, SPSS 14.0 was used. To examine correlations, Pearson correlation analysis was used; to test the significance of different series, Fisher's exact test (with two methods) was used; and to compare the groups, one-way ANOVA was applied. The results were accepted as statistically significant at $P < 0.05$.

## 3. Results

The distribution of the cases according to tumor stages was T1: 15, T2: 12, T3: 1, and T4: 4. Fourteen patients had pure seminomas, 18 patients had nonseminoma germ cell tumors (NSGCTs), only one had embryonal carcinoma, and the rest had mixed components. The maspin-positive staining pattern was examined in tumor cells in the cytoplasm and the nucleus. No nuclear staining was detected. Although only 1 out of 14 seminoma cases was positive for maspin, 6 out of 18 NSGCTs were positive for maspin (Figures 1A and 1B). The stages were evaluated in terms of maspin positivity by setting up binary groups among them, and no difference was detected statistically between the stages. The P-values were T1–T2, $P = 0.05$; T1–T4, $P = 0.39$; and T2–T4, $P = 0.18$. No further statistics were produced since there was only 1 patient with aT3 stage tumor. The patients' tumor sizes were separated into two groups: ≤4 cm and ≥4 cm. The relation between maspin and tumor size was assessed for the two groups. No statistical correlation was detected ($P > 0.05$). Serum AFP and HCG values of the mixed GCT cases were studied. All patients' HCG values were below 5000 mIU/mL when classified in accordance with the EAU guidelines. Although the AFP values for 14 AFP-positive patients were below 1000, in two patients, the values were between 1000 and 10,000, and in one patient, the value was above 10,000. The statistical analysis showed no correlation between maspin positivity and AFP ($P > 0.05$).The patients' HCG values were below 5000 mIU/mL, and no statistically significant relation was determined between maspin positivity and HCG.

The positive staining pattern for VEGF was accepted as cytoplasmic positive staining in tumor cells. In 8 cases of seminomas, less than 5% staining occurred or no staining took place at all. In the cases of NSGCTs, in 11 patients, there was more than 50% (2+) staining. No significant relation was detected between maspin and VEGF in patients with seminomas ($P > 0.05$). Similarly, no relation was determined between maspin and VEGF in NSGCTs ($P > 0.05$).

In p53 evaluation, the positive staining pattern was nuclear (Figures 1C and 1D). In 3 seminoma cases, p53 stained positively in more than 50% of the cells; however, more than 50% staining occurred in 10 of the NSGCT cases. No staining occurred in one seminoma case. This was the only maspin-positive case among the seminomas. No statistically significant difference was detected between maspin and p53 in cases of seminoma ($P > 0.05$). In the statistical analysis conducted on NSGCTs, there was a positive correlation between maspin and p53 ($P < 0.001$).

In the evaluation of Ki-67, the positive staining pattern was nuclear positive staining in tumor cells. There was 35.6% staining in the seminomas while there was 50.1% staining in the NSGCTs. No statistical connection was established between maspin and Ki-67 in the seminoma cases ($P > 0.05$). The relation between maspin and Ki-67

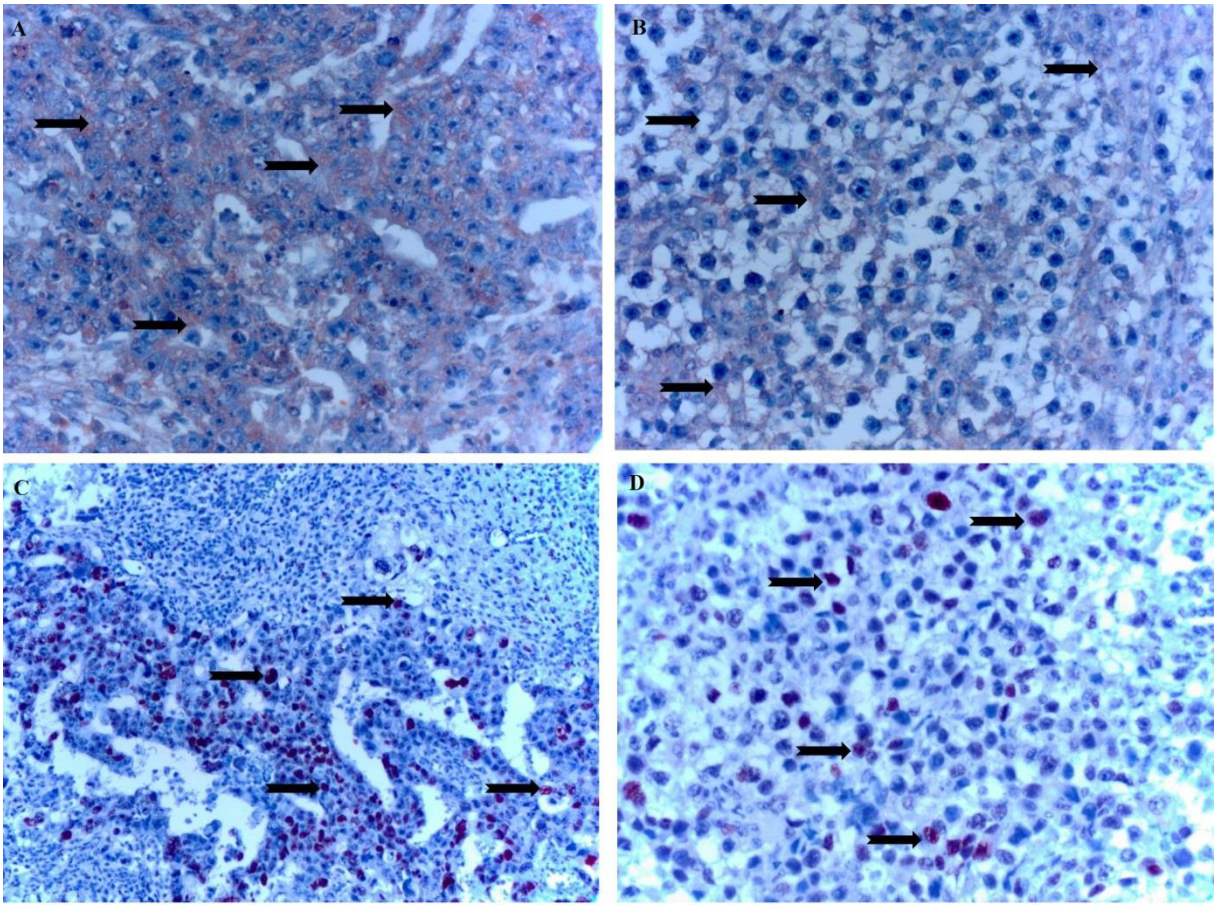


**Figure 1.** Cytoplasmic brown staining positivity in embryonal carcinoma (A) and seminoma (B) areas and nuclear brown staining positivity in embryonal carcinoma (C) and seminoma (D) areas for p53.

was statistically insignificant in the NSGCTs ($P > 0.05$).

In CD31 evaluation, the mean vein count was calculated in five large magnification areas (5-HPF, 400× magnification). The mean CD31 of the seminoma cases was 12.2, whereas the mean CD31 of the nonseminomatous cases was 8.8. No statistical difference was found between maspin and CD31 in either seminoma or nonseminomatous tumors ($P > 0.05$).

## 4. Discussion

Maspin, which was first discovered in breast tissue and breast cancer cell lines with the hybridization procedure, is decreased in invasive and metastatic breast and prostate cancers (1,2). The tumor suppressor function of maspin was shown in in vitro studies. Unlike cell adhesion and other serpins, maspin leads to an increase in apoptosis and a decrease in cell motility, angiogenesis, and pericellular proteolysis (1,4). In some experimental studies, maspin prevented the development and/or progression of malignant tumors with the p53-dependent pathway, plasminogen activation inhibition, and angiogenesis inhibition (11). Studies also suggested that maspin takes effect through angiogenesis and is associated with VEGF (12). High maspin expression was shown in normal human breast and prostate epithelium cells. However, expression decreased in the cancer cells of these organs and disappeared in metastases (3–5). Other studies suggested that increased maspin expression was a good prognostic factor in oral squamous cell carcinomas (4). In contrast, increased maspin expression was detected in pancreas-origin cancers, whereas no maspin expression was observed in normal pancreas cells (11).

Various reports have suggested that nuclear and cytoplasmic maspin expression has different clinicopathologic importance in different types of tumors. That nuclear positivity was a good prognostic indication was emphasized in some studies, while in others maspin positivity was associated with shorter-term survival. Sood et al. reported that cytoplasmic localization was related to a poor prognosis, whereas nuclear localization was associated with benign cases in ovarian cancers (3). In a study conducted on adenocarcinomas, improved

morphologic indications were found in patients who showed nuclear maspin positivity (13). Nakagawa et al. did not determine a difference between maspin-positive and -negative groups in terms of prognosis (11). However, the researchers suggested that cytoplasmic-stained maspin was a good prognostic factor in renal tumors (7). Maspin positivity was present in only 1 out of 14 seminoma cases and 6 out of 18 NSGCT cases in our study. All maspin-positive cases were cytoplasmically stained, and no nuclear staining was observed in any tumors. Due to the small number of positively stained tumor cells, it is hard to say whether cytoplasmic staining is an indication of a good or bad prognosis. Perhaps different subforms (active or inactive) of maspin, which was detected immunohistochemically in the cytoplasm, could exist at the molecular level, and these forms might reveal the behavior of maspin in tumors.

Maspin positivity in NSGCTs was significantly higher than in the seminoma group in our study. Prognosis for NSGCTs was worse than that for seminomas. No connection was observed between maspin and tumor stage, tumor size, or AFP level. Thus, maspin is not a predictor of a bad prognosis in testicular tumors, but the fact that it exists more in NSGCTs is an important subject that should be investigated in wider chain studies.

Along with tumor angiogenesis, VEGF functions as an autocrine and paracrine growth factor that induces the proliferation of tumor cells. A connection between VEGF levels and metastases and/or bad prognosis was found in many tumors within the body (12–14). Studies in the literature regarding VEGF and testicular tumors are lacking. VEGF expression was shown to be significantly higher in testicular tumors in a study carried out by Fukuda et al. and in multivariate analyses it was reported that VEGF displayed significant correlation with metastases development, especially in seminomas (15). The high percentage of VEGF staining in our study agreed with the findings in the literature. Although studies have indicated a significant relation between maspin and angiogenesis, this relation was not observed in our study. Likewise, no relation was discovered between maspin and VEGF in a previous study conducted on renal tumors (7). Maspin, as is the case with renal tumors, may have had an effect at the cellular level with a nonangiogenesis mechanism different from other tumor groups. Ki-67 is a proliferation kinetics index used to determine the correct histopathologic diagnosis, prognosis, and treatment approaches in many malignant tumors (16).

The Ki-67 index in germ cell tumors in testes is used to determine a patient's risk group. Düe et al. investigated the Ki-67 antibody and the growth pattern of tumors and proliferative activity immunohistochemically in 20 seminoma cases and found that the growth was between 50% and 80%. In this study, in which most of the patients' pathologic stages were advanced, there was a relation between the tumor pathology and the proliferation rate. Since the proliferative activity of seminomas determined their sensitivity to chemotherapy and radiation, it is advised to look for Ki-67 in these tumors (17). The Ki-67 index of the patients with seminomas was 35.6 and that of NSGCTs was 50.1 in our study. Statistical analysis showed no connection between maspin and Ki-67 ($P > 0.05$).

The p53 tumor suppressor gene located on the short limb of the 17th chromosome (17pl31) has many mutations (18). p53 overexpression and mutations can be seen in many cancers with little differential and associated poor prognosis. Bostwich et al. reported that p53 immunoreactivity in urothelial carcinomas was associated with high tumor stage, degree, vascular invasion, recurrence, and progression (19). Since p53 expression usually coexists with resistance to radiotherapy and chemotherapy and the sensitivity of testis GCTs to radiotherapy and chemotherapy, whether p53 protein expression in GCTs is dependent on a mutation occurring in the p53 gene has been investigated in many studies (20,21). Although studies have reported that p53 mutations occur in testicular GCTs, other studies have reported that the protein expressed in these tumors is a wild-type p53 protein (19–21). p53 expression in normal cells suggests a good prognosis. However, p53 overexpression and mutation in tumor cells are associated with a poor prognosis (18). p53 staining of seminomas and NSGCTs was evaluated separately in this study. Strong p53 expression was detected in NSGCTs compared with seminomas. This result is important in terms of indicating that mutant p53 was expressed much more than in NSGCTs with a more aggressive course. In addition, a statistically positive relation between maspin and p53 in NSGCTs was demonstrated. Maspin may have displayed an effect through the p53 pathway in NSGCTs.

CD31 is a molecule in an immunoglobulin superfamily weighing 130 kDa and generally one of the most widely used markers for measuring vein density, which is the arithmetic measurement of tumor angiogenesis (7). In a study carried out by Yilmazer et al., CD31 and CD34 markers were used for measuring microvein density (MVD) in renal cell carcinomas (RCCs). Although a significant relation was detected between CD34 staining and tumor progression, no correlation between CD31 level and tumor type, stage, nuclear degree, or survival was observed (22). However, Turunc et al. showed that MVD measured with the CD31 marker was in counter-relation to the progression of RCCs and reported that it was a good prognostic factor (7). The vein count in five large magnification areas for CD31 was calculated in our study, and no statistically significant relation was found between maspin and CD31 in either seminomas or NSGCTs.

In conclusion, that maspin was expressed in testicular tumors was first shown in this study. Maspin was expressed more in NSGCTs compared with seminomas, but no relation was observed between maspin and tumor stage and size. Thus, it remains unclear whether maspin is a poor prognostic factor in testicular tumors. Moreover, no connection was observed between maspin and VEGF, Ki-67, or CD31. Therefore, maspin might have affected testicular tumors through a different method or receptor at the cellular level apart from angiogenesis or similar pathways. Thus, the fact that a significant relation was observed between maspin and p53 makes us think that maspin could have an effect via the p53 pathway. To discover the mechanism of action of maspin in testicular tumors, wider chain studies are needed.

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