JBUON 2018; 23(4): 1125-1129
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

N-cadherin expression in primary and metastatic testicular germ cell tumors

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Summary

Purpose: *Upregulation of N-cadherin in epithelial tumor cells has been reported to enhance the invasive process. Although the distribution of N-cadherin in the normal testis was demonstrated, there is no adequate information regarding its presence in testicular germ cell tumors (GCTs). Our purpose was to examine the expression and localization of N-cadherin in germ cell tumors of the testis and share our experience.*

Methods: *104 adult cases of primary and metastatic testicular GCTs, 5 germ cell neoplasia in situ and 15 benign testicular tissues were included into the study and analyzed for immunoexpression of N-cadherin. Positive expression was evaluated according to intensity and localization of the staining.*

Results: *In 34 pure seminomas, 6 seminomas of mixed component and 16 metastatic seminomas, N-cadherin expression was observed with variable staining intensity. Two pure yolk sac tumors and 20 out of 34 mixed GCTs with yolk sac components were positive for N-cadherin. In contrast, neither the embryonal and chorionic carcinomas nor the teratomas showed N-cadherin expression.*

Conclusions: *N-cadherin immunexpression should be interpreted considering both staining intensity and extent. In case of a metastatic tumor of unknown primary showing prominent N- cadherin expression, seminoma should be considered in the differential diagnosis. At the same time N-cadherin staining could be used to differentiate seminomas from embryonal carcinomas with solid components in metastatic GCTs, both having similar histopathological findings. On the other hand, the diagnostic value is not obvious for nonseminomatous tumors.*

Key words: *germ cell tumor, immunohistochemistry, N-cadherin, testis*

Introduction

Cadherins are specialized membrane glycoproteins and play an important role in many biological processes such as cell-cell contact, cell signaling, differentiation, embryonic development and tumorigenesis [1,2]. More than 80 different members constitute the group of cadherins. Epithelial (E), placental (P), neuronal (N) and retinal (R)-cadherins are the best investigated of all [3,4]. N-cadherin was originally identified as a cell adhesion molecule expressed in neural tissues, but it has been shown to be expressed in various non-neural tissues, such as thymus, kidney [5], pancreas and liver [6,7]. Although the distribution of N-cadherin in the normal testis is known, expression of N-cadherin in GCTs of the testis has not been well studied. In this study, we examined the expression and localization of N-cadherin in a series of malignant GCTs of the testis, germ cell neoplasia *in situ* (GCNIS), and normal seminiferous tubules.

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Received: 02/01/2018; Accepted: 30/01/2018

Methods

One hundred and four GCTs were retrieved from the database of our hospital between in 2005-2014. The tumors were classified according to the 2016 World Health Organization classification [8]. Hematoxylin and eosin stained slides of the tumors were reviewed and representative blocks were selected for immunohistochemical staining. A monoclonal antibody against N-cadherin (DAKO, Hamburg, Germany) was selected and streptavidin-biotin methodology was used for immunohistochemical staining. Tissue sections were deparaffinized with overnight incubation at 60°C, rehydrated, and then boiled in a microwave oven for 20 min at 95°C, equivalent to 750 watts. Following incubation with 3% hydrogen peroxide, the sections were kept at protein blocking antibody for 10 min and then incubated with the primary antibody for one hr at room temperature. Then, they were incubated with anti-rabbit biotinylated secondary antibody and streptavidin-HRP for one hr and 15 min, respectively. Subsequently, diaminobenzidine (DAB) chromogen solution was applied for 10 min. After counterstaining with hematoxylin, the sections were dehydrated and cleared. Immunoreactivity was evaluated under light microscope. All tissue sections were evaluated considering staining of N-cadherin at membranes and the cytoplasm.

The percentage of positively stained cells was first categorized using a 0–4 point scoring system: Score 0 = no positive cells, score 1 <25% positive cells, score 2 26-50% positive cells, score 3 51-75% positive cells, and score 4 >76-100 % positive cells. The intensity of staining was evaluated on a graded scale 0: negative; 1: weak; 2: intermediate; and 3: strong.

Statistics

The statistical data were analyzed using the SPSS 22 (SPSS, Chicago, Ill, USA). Comparisons between N-cadherin expression patterns and tumor types were evaluated using Fisher exact test and Fisher-Freeman-Halton exact test. A p value<0.05 was considered as statistically significant.

Table 1. Distribution of pure and mixed GCTs included in the study

Tumor type	*Testicular tumors (n)*	*Metastatic tumors (n)*	*Total (n)*
Pure GCTs			
Seminoma	36	16	52
Teratoma	9	5	14
YST	2	-	2
EC	3	2	5
Mixed GCT	30	1	31
Total	80	24	104
Mixed GCTs			
Teratoma	23	1	24
EC	16	1	17
YST	18	1	19
Seminoma	15	-	15
CC	9	-	9

CC:choriocarcinoma, EC:embryonal carcinoma, GCT:germ cell tumor, YST:yolk sac tumor

Table 2. N-cadherin expression in pure, mixed germ cell tumors and metastatic tumors

	No	*Tumor cells positivity (%)*					*Intensity*		
Pure tumors		0	1-25	26-50	51-75	76-100	1+	2+	3+
Seminoma	52	5	2	3	16	26	20	22	10
YST	2	1	1	-	-	-	-	2	-
EC	5	5	-	-	-	-	-	-	-
Teratoma	14	14	-	-	-	-	-	-	-
Mixed tumors									
Seminoma	15	9		3	2	1	5		1
YST	20	3	2	1	5	9	1	13	6
EC	17	17	-	-	-	-	-	-	-
Teratoma	24	24	-	-	-	-	-	-	-
CC	9	9	-	-	-	-	-	-	-

For abbreviations see footnote of Table 1

Results

Our series of malignant GCTs of the testis included 36 (34.6%) cases of pure seminomas, 2 (1.9%) pure yolk sac tumors, 3 (2.9%) pure embryonal carcinomas, 9 (8.7%) pure teratomas and 30 (28.9%) mixed GCTs with various histologic components (Table 1). Among these tumors, 24 cases (16 pure seminomas, 5 pure teratomas, 1 mixed GCT and 3 embryonal carcinomas) were diagnosed with a lymph node or another organ metastasis. Five cases of GCNIS and 15 cases of benign testicles with normal seminiferous tubules were also included in the study. For the differential diagnosis of GCTs in our study we used the panel of immunohistochemical markers i.e. CD30, Glypican-3, β-HCG, AFP. The percentage of positively stained cells and intensity of staining for N-cadherin in tumors of pure, mixed histological type and metastatic lesions are seen in Table 2. In the normal testis, N-cadherin was consistently seen in a membranous pattern in Sertoli cells. It was also detected in similar pattern in the rete testis. Interstitial and Leydig cells were completely negative for N-cadherin. GCNIS stained strongly with N-cadherin within the cytoplasm and more obviously at the cell membrane (Figure 1). N-cadherin was observed in 76-100% of cells with 3+ intensity in the pure seminomas, mixed germ cell tumors with seminoma components and metastatic seminomas. The expression was located only at areas of cell to cell contact and membrane-bound (Figure 2). Two pure yolk sac tumors and 20 yolk sac tumor components out of 34 mixed GCTs were positive for N-cadherin (Figure 3). The staining was mainly cytoplasmic and lesser membranous. The staining intensity was 2+ in 51-75% of the cells. In contrast, N-cadherin could not be detected in pure embryonal carcinomas, mixed tumors and metastatic tumors. Chorionic carcinoma components within GCTs also proved to be negative for N-cadherin expression. Similarly, pure teratomas, mixed tumors with teratomas and metastatic teratomas were negative for N-cadherin.

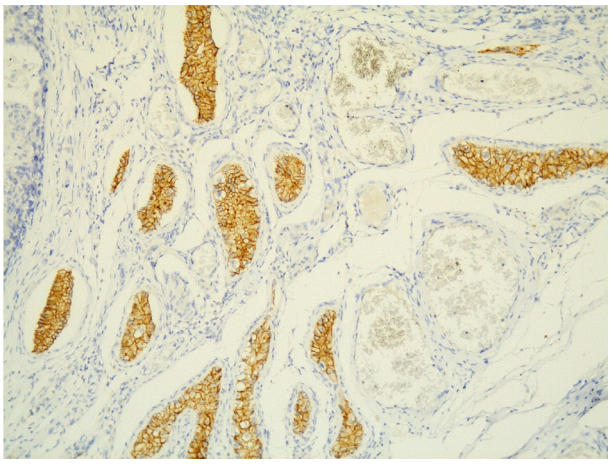

Figure 1. Intratubular germ cell neoplasia strongly expressing N-cadherin.

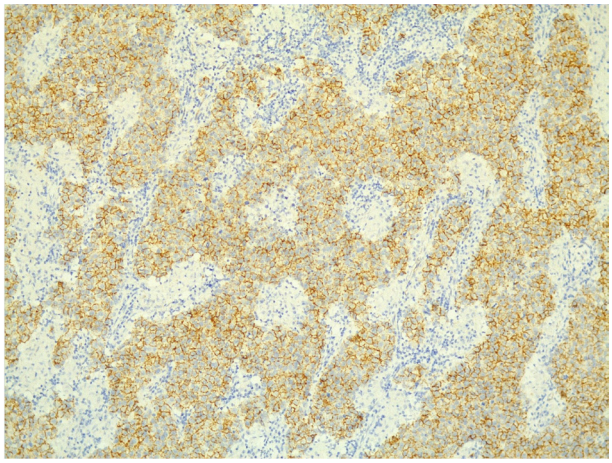

Figure 2. Seminoma showing strong N-cadherin expression.

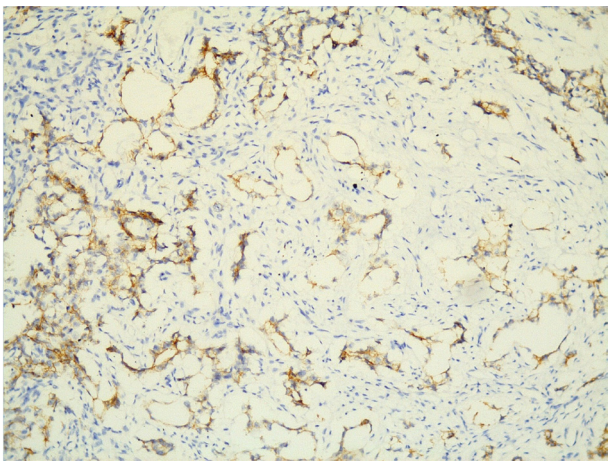

Figure 3. Yolk sac tumor showing N-cadherin expression.

Discussion

In carcinogenesis, tumor cells show decreased cell–cell adhesion, increased motility, invasion of basement membranes, intravasation of lymphatics and blood vessels, and extravasation at distant sites [9-11]. One of the steps in the invasive or metastatic process is loss of E-cadherin expression and upregulation of N-cadherin. Recent studies have revealed that the physiological function of N-cadherin in adult tissues is not only important in maintaining the adhesive function [1,12,13] but also its expression in endothelial cells plays an essential role in the maturation and stabilization of normal and tumor-associated angiogenic vessels [14]. Currently there is increasing experimental evidence suggesting that N-cadherin is a potential therapeutic target in many cancer types [13].

N-cadherin expression and its relationship to invasion have been reported in several cancer cell lines. In the literature, N-cadherin is reported to show *de novo* expression, re-expression, upregulation, or downregulation in many cancer types [15]. For example, neoexpression of N-cadherin has been demonstrated in gastric [16], esophageal [17], pancreatic [18] and prostatic tumors [19]. As all melanoma cells and cells of α-fetoprotein-producing tumors, embryonic precursor cell derived tumors have re-expression of N-cadherin [20-22]. In our study, staining patterns of seminoma and yolk sac tumors were consistent with re-expression pattern. In cancer groups of breast [23], prostate [24], colon [25], and pancreas [18] N-cadherin shows upregulation. The cells already expressing N-cadherin in embryonic and adult stages can already increase their expression levels in neoplastic stages. Downregulation of N-cadherin has been shown in osteosarcoma and disseminated malignant astrocytic tumors [26,27]. However, N-cadherin has been found to correlate strongly with tumor aggressiveness in all tumor types.

In the literature, the studies investigating N-cadherin expression of nonneoplastic or neoplastic testicular tissue are very rare. According to Tsuchiya et al., N-cadherin immunoreactivity is present in the seminiferous epithelium including spermatogonia, primary spermatocytes and Sertoli cells in the testis. No E-cadherin immunoreactivity has been detected in the testis except for the epithelium of the efferent ducts. In the epididymis, E-cadherin has only been detected in the epithelial cells [28]. Similarly, Andersson et al. has demonstrated N-cadherin immune expression on the surface of spermatogonia, primary spermatocytes, and also around some early spermatids in the testis [29]. These observations indicate that N-cadherin plays an important role in the organization of the cells in the seminiferous tubules. Bremmer et al. has presented the largest case series with 113 patients on N-cadherin expression of testicular tumors, indicating that seminoma has N-cadherin expression but embryonic carcinomas and components of chorionic carcinoma have not [30]. In their study, they have observed cytoplasmic and membranous N-cadherin expression in tumor-free testis, GCNIS, seminomas, yolk sac tumors and in primitive neuronal elements within teratomas.

GCNIS cells are derived from primordial germ cells and transform into GCT after puberty. GCNIS is the common precursor of seminomas and nonseminomatous tumors. Seminomas have limited capacity to differentiate into somatic or extra-embryonic tissues, although they can switch to a nonseminomatous phenotype [31]. In particular, they may reprogram into an embryonal carcinoma cell. Embryonal carcinoma cells are the stem cells of non-seminomas and can rise to embryonic endo-, meso- and ectoderm and/or differentiate into extra-embryonal yolk sac and trophoblast. But they have never been shown to rise to the germ line in humans [32]. Yolk sac tumors may exhibit germ cell lineage differentiation [33]. Therefore N-cadherin staining in these tumors may occur.

Our study has similar results with the Bremmer et al.study, except for our observation of mainly membranous N-cadherin expression in benign testicular tissue. We found that N-cadherin was associated with seminoma and yolk sac tumor types. Staining was very obvious at cell borders. Yolk sac tumors showed both cytoplasmic and membranous staining pattern. The staining pattern in seminoma and yolk sack tumor may help the diagnosis of metastatic GCTs and should be included in the panel of immunohistochemical markers, especially in metastatic tumors of unknown origin. At the same time N-cadherin staining can be useful for differentiating seminomas from embryonal carcinomas with solid components in metastatic GCTs having similar morphological appearance.

In the Bremmer's study, N-cadherin staining in neuronal elements was positive in pure and metastatic teratomas. In our study, teratomas were predominantly consisted of epithelial and mesangial elements, lacking neuronal components, so we were unable to show N-cadherin expression. We did not observe any staining in metastatic mixed GCTs with components of embryonal carcinoma and yolk sac tumor. Owing to the very few numbers of non-teratomatous metastatic tumors, N-cadherin expression in the metastatic group could not be evaluated correctly and in detail.

Conclusion

Several immunohistochemical markers are known for the differential diagnosis of GCTs. N-cadherin expression on GCTs of the testis is under investigation and it is proved to be useful for the differential diagnosis of seminoma and nonseminomatous GCTs, especially for embryonal carcinomas with solid components.

Conflict of interests

The authors declare no conflict of interests.

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