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Low Expression of DNA Polymerase β in Human Testicular Germ Cell Tumours

Impact on Foetal Gonocytic Origin Theory

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Different models of pathogenesis of adult testicular germ cell tumours (TGCTs) are presented. Analysis of telomeric length and DNA polymerase β expression suggests that seminoma and nonseminoma, two main histological types of TGCTs, derive independently from transformed foetal primordial cells.

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Testicular germ cell tumours (TGCTs) are the most common cancers affecting young men. Increasing risk of testicular cancer has been observed in many countries (1–3). In Poland, standardized incidence rates in 1973, 1983 and 1996 were 1.2, 1.8, and 2.7, respectively (4, 5). Approximately 95% of testicular tumours are of germ cell origin (6). These tumours have a characteristic chromosome pattern with a high copy number of the short arm of chromosome 12 (12p). The gene locus of the homotetramer subunit of LD-1 has been localized to 12p, making LD-1 a useful marker in the staging and monitoring of patients with TGCT (7). There are three main histological subtypes of TGCTs: seminoma, nonseminoma and a rare spermatocytic seminoma also known as spermatocytoma (6). The most frequent tumours—seminoma and nonseminoma—arise from a common premalignant lesion, which consists of large, intratubular, gonocyte-like cells with large irregular nuclei and vacuolated cytoplasm containing abundant glycogen (8). This precursor lesion, termed carcinoma-*in situ* (CIS) or intratubular germ cell neoplasia (ITGCN), can be found in the adjacent parenchyma of most TGCTs. One of the main issues discussed at the first Copenhagen Workshop on Carcinoma *in Situ* in 1980 was the question of the actual existence of CIS in the testis. Twenty years later, nobody doubts the existence of CIS, but the problem of the precursor cell of CIS remains.

There are two hypotheses about the origin of CIS, one of which states that CIS cells derive from foetal primordial cells early in embryogenesis as a consequence of endocrinological imbalance (for a review, see (9, 10)). Epidemiological data as well as phenotypical similarities of primordial germ cells and CIS cells endorse this view. Both types of cells share expression of placental-like alkaline phosphatase (PLAP) and the stem-cell factor receptor, c-KIT. Biallelic expression of imprinted genes in seminoma and nonseminoma also supports the hypothesis that TGCTs, with the exception of spermatocytic seminoma, originate from germ cells which had undergone early erasing of imprinting (11). The paternal pattern of genomic imprinting seen in spermatocytic seminoma suggests that this rare germ cell tumour originates from spermatogonia B (9). However, the recent detection of c-KIT positivity in some spermatocytic seminoma samples indicates that at least some of these tumours may also originate from primordial cells (12).

The second hypothesis postulates that the precursor lesion—CIS, results from tetraploid arrest and malignant transformation of pachytene primary spermatocytes (13). This proposal is based on the following properties of TGCTs: increased 12p copy number, expression of cyclin D2 in CIS, near triploid-tetraploid chromosome numbers,

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and over-expression of wild-type p53. This last feature is based on murine studies (14), which have shown that in the pachytene stage of gametogenesis, there is a high level of expression of p53 (mRNA and protein), which most likely allows for the arrest of the cell cycle and facilitates recombinational repair of DNA breaks. As we have previously shown (15, 16), DNA polymerase β, the expression of which is regulated mainly on the mRNA level (17), is yet another protein that is over-expressed in pachytene spermatocytes. This DNA repair enzyme is expressed constitutively in all cells at a low level. However, the very high level of DNA polymerase β mRNA observed in pachytene spermatocytes points to the role of this enzyme in mammalian meiosis. Antibody localization data have confirmed that polymerase DNA β indeed participates in meiotic events associated with synapsis and recombination (18).

In this study we examined the expression pattern of DNA polymerase β in TGCTs. If these tumour cells originate from meiotically arrested pachytene spermatocytes, it should be expected that DNA polymerase β mRNA would also be over-expressed in them. To verify this, we used Northern blot analysis of the single human pol-β transcript of 1.4 kb.

MATERIAL AND METHODS

Tissue samples

Fresh surgical specimens of human testicular tumours and testis of prostate cancer patients who had undergone orchidectomy were obtained from the Pathology Department of the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, as part of the material sent from the surgery unit. Samples were immediately frozen in liquid nitrogen and stored at − 70°C. The tumours studied included two cases of seminoma, two cases of embryonal carcinoma, one case each of mixed teratoma/embryonal carcinoma, mixed seminoma/embryonal carcinoma, spermatocytic seminoma and non-germ cell tumour.

Northern blot analysis

Total RNA was prepared according to Chomczynski & Sacchi (19). Poly $(A)^+$ mRNA was isolated by means of a single passage through an oligo(dT) column (20). This procedure produced samples containing approximately equal quantities of polyadenylated and non-polyadenylated species of RNA (20), enabling the estimation of gel loading. RNA concentration was determined spectrophotometrically. RNA integrity was checked electrophoretically. RNA electrophoresis and hybridization to the DNA polymerase β probe was done as described elsewhere (15).

Densitometric analysis of autoradiograms

Autoradiograms were scanned and analysed using the OptiQuant 3.10 Software Package (Packard BioScience Company). Band intensities from Northern blots were measured in DLU (Digital Light Units)/mm^2 and calculated in reference to 18S ribosomal RNA quantities for each respective sample, obtained in the same units.

These relative amounts were further normalized against the sample with total depletion of seminiferous epithelium, separately for each experiment.

RESULTS AND DISCUSSION

An example of Northern blot analysis of poly $(A)^+$ RNA isolated from testicular tumours is shown in Fig. 1. Poly $(A)^+$ RNA samples from the testes of prostate cancer patients who had undergone orchidectomy acted as controls. In the seven control samples, the level of DNA polymerase β expression was reciprocally proportional to the progression of testis atrophy and depletion of seminiferous epithelium (see Table 1). The level of either DNA polymerase β or p53 mRNA in the seven germ cell tumours tested and one non-germ cell tumour was no higher than that in the control samples (see Table 1). It can therefore be concluded that DNA polymerase β is not abundantly expressed in TGCTs. Overexpression of p53 on the protein level is often observed in TGCTs (21). Our findings indicate, however, that as opposed to pachytene spermatocytes, this is not the case on the mRNA level.

Recently, expression of $p19^{INK4d}$, a member of the INK4 family of cyclin-dependent kinase inhibitors, which negatively regulates cyclin D/CDK4(6) complexes, has been examined in normal human testis development and in testicular cancers (22). The authors found that this protein is abundant in spermatocytes but is not detectable either in

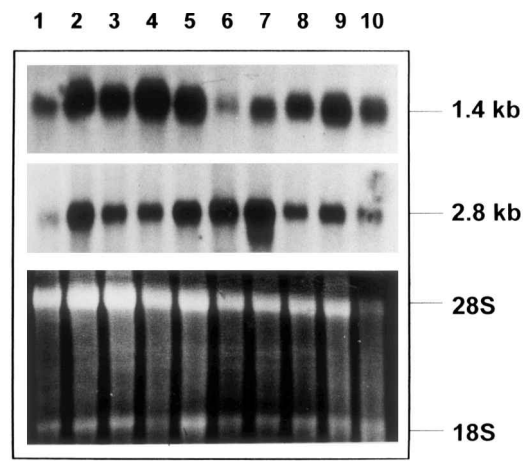

Fig. 1. Northern blot analysis of poly $(A)^+$ RNA from testis of prostate cancer patients (lanes 1-5) and testicular tumours: spermatocytic seminoma (lane 6), mixed teratoma/embryonal carcinoma (lane 7), seminoma (lanes 8 and 9), mixed seminoma/embryonal carcinoma (lane 10). The samples were electrophoresed, blotted, and hybridized with β-pol probe (upper part); p53 probe (middle part) as described previously (15). Gel loading was estimated by ethidium bromide staining of 18S rRNA.

Table 1

Relative expression of DNA polymerase β and p53 in reference to 18S rRNA. Results normalized against sample with total depletion of seminiferous epithelium

No. of samples	Control samples								Tumour samples							
	1 Lane 1*	6 Lane 3	9	2	3 Lane 2	5 Lane 5	7	8 Lane 4	4 Lane 6	10 Lane 7	11 Lane 8	12 Lane 9	13 Lane 10	14	15	16
Status of spermatogenesis**	–	–	–	+	+	nd	+	+								
Pol-β	1	1	1	2.9	1.8	0.9	2.9	2.4	0.3	0.6	0.8	1.3	2.2	0.2	0.5	0.9
P53	1	0.8	1	1.1	1.5	1	1.3	1.4	1.5	1.9	0.5	0.9	1.3	0.5	1.3	1.2

* Number of the lane in Fig. 1.
** – No spermatogenesis; + remnant spermatogenesis; nd = not determined.

human foetal germ cells or at any stage of germ cell tumour pathogenesis. Similarly, RBM (RNA binding motif) protein is expressed exclusively in spermatogonia, spermatocytes and round spermatids, while TGCTs are completely immunonegative for RBM (23). Both, the immunohistochemical and molecular approach show that SSX (synovial sarcoma on X chromosome), SCP1 (synaptonemal complex protein 1) and XPA (xeroderma pigmentosum type A) are specifically present in pachytene spermatocytes, while CIS and seminoma cells are devoid of protein encoded by these genes (24). A low level or absence of expression of DNA polymerase β mRNA, p53 mRNA, $p19^{INK4d}$, RBM, SSX, SCP1 and XPA in human TGCTs contrasts with high expression of these proteins during spermatogenesis, thus rendering the theory of pachytene spermatocitic origin of TGCTs doubtful. Another argument that supports an 'embryonic state' of neoplastic germ cells is based on a recent observation of retained foetal expression of angiotensin I-converting enzyme (CD143) as well as checkpoint protein Chk2 in CIS and TGCT (25, 26). All these data support the concept of the foetal origin of adult TCGTs.

The pathogenetic relationship between seminoma and nonseminoma also remains a matter of debate. The linear progression model based on DNA ploidy, cytogenetic and LOH data postulates clonal 'reprogramming' of seminoma into nonseminoma by the accumulation of genetic losses (27, 28). Another model is based on the observation that the relative incidence of nonseminoma decreases with age and assumes that both subtypes of TGCTs derive independently from CIS. Seminoma may originate from 'aged' CIS cells that have little or no stem-cell potential, whereas nonseminoma may originate from 'young' CIS cells that have conserved their totipotential stem-cell capacity (29). Our recently published data (30) on comparison of telomeric length between seminoma and nonseminoma have shown that germ cell-like telomere length homeostasis is preserved only in nonseminomatous TGCTs. This means that seminoma cells lose while nonseminoma cells retain their totipotential stem-cell behaviour according to the divergent pathways model (29).

We conclude that although one cannot exclude the pachytene spermatocytic origin of TGCTs, the majority of data, including our results on the expression of DNA polymerase β, support the hypothesis that it is foetal gonocytes that are target cells for transformation. In addition, our earlier study of telomeric length endorses the model in which two main histological types of TGCTs derive independently from the precursor cell.

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