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Automated image analysis DNA cytometry in testicular cancer

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Abstract The value of automated DNA cytometry for differentiation of testis cancer was evaluated in 54 seminomas, 13 HCG-positive seminomas, and 48 embryonal carcinomas. Slices of paraffin embedded tissue were enzymatically digested and stained with Feulgen SITS after fixation on glass slides. Automated DNA cytometry was performed with a Modular Image Analysis Computer (MIAC). DNA histogram phenotpye and computed DNA indices were correlated with the different tumor types. The ratio of hypertriploid to hypotriploid increased from HCG-positive seminoma over embryonal carcinoma to seminoma. The following mathematical DNA indices were found to correlate with tumor type: mean ploidy, 2c deviation index, 5c exceeding rate, variation coefficient of the GO/1 fraction and DNA nucleus diameter correlation.

Key words Testicular tumor · Automated image analysis · DNA cytometry · Classification · Seminoma · Embryonal carcinoma

Cytophotometric determination of the DNA content in cell nuclei has frequently been regarded as a diagnostic and prognostic tool in various tumors. In the urogenital system, most investigators have focused on tumors of the prostate [17, 22], kidney [14, 16, 21] and bladder [13, 20]. Since testicular cancer is a relatively rare tumor type, DNA cytometric studies have been done only in a limited number of patients with this malignancy.

Most quantitative studies on nuclear DNA in testicular germ cell tumors revealed an aneuploid pattern [2, 7, 12]. However, there is no general agreement on the percentage of diploid testicular tumors since this percentage varies from 5% to 30% in the literature [2, 8, 12, 18]. There is also no general consensus with regard to the differences in DNA content between seminomas and non-seminomatous germ cell tumors [8, 15, 23].

We analyzed DNA ploidy patterns in various types of testicular germ cell neoplasms (seminoma, HCG-positive seminoma, and embryonal carcinoma) by the use of automated DNA image analysis cytometry.

Materials and methods

Paraffin-embedded histological material obtained by orchiectomy from 115 patients with testicular cancer was analyzed (Table 1). Tumor areas within the paraffin material were identified by HE-stained reference sections. One 50-µm section was deparaffinated, rehydrated in ethanol at decreasing concentrations, digested in a solution of 0.05% pronase for 30 min, resuspended mechanically by repeated sucking through Pasteur pipettes, and filtered over 70-µm nylon mesh filters. The cells were centrifuged and resuspended in carbowax fixative and adjusted to a concentration of 10^4 cells/ml. Then 1 ml of this suspension, containing bare nuclei with minimal rests of cytoplasm was centrifuged on glass slides. The nuclei were air-dried and stained by the Feulgen-SITS technique with an automatic staining device. Healthy testicular tissue was processed by the same techniques and served as a diploid standard.

Automated analysis of Feulgen-DNA-stained testicular specimens was performed with a Modular Image Analysis Computer (MIAC; Leitz, Wetzlar, Germany). This machine consists of a computer-operated microscope and two video cameras. Image analysis is performed by the Texture Analysis System (TAS; Leitz). The digitized images are stored on a personal computer. The MIAC is operated by automatic cell analysis program (ACA, Microscan,

Table 1 Tumor histology and pT stages of the tumors analyzed

	Seminoma $n=54$	HCG-pos. $n=13$	Embryonal carcinoma $n=48$
pT1	36	9	25
pT2	14	3	16
pT3	4	1	5
pT4	0	0	2

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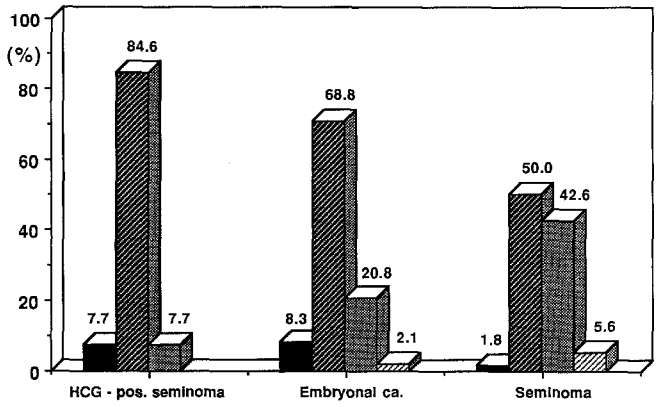

Fig. 1 Ploidy of the stem cell lines. ■ Diploid; ▨ hypotriploid; ▩ hypertriploid; ▨ hypertetraploid

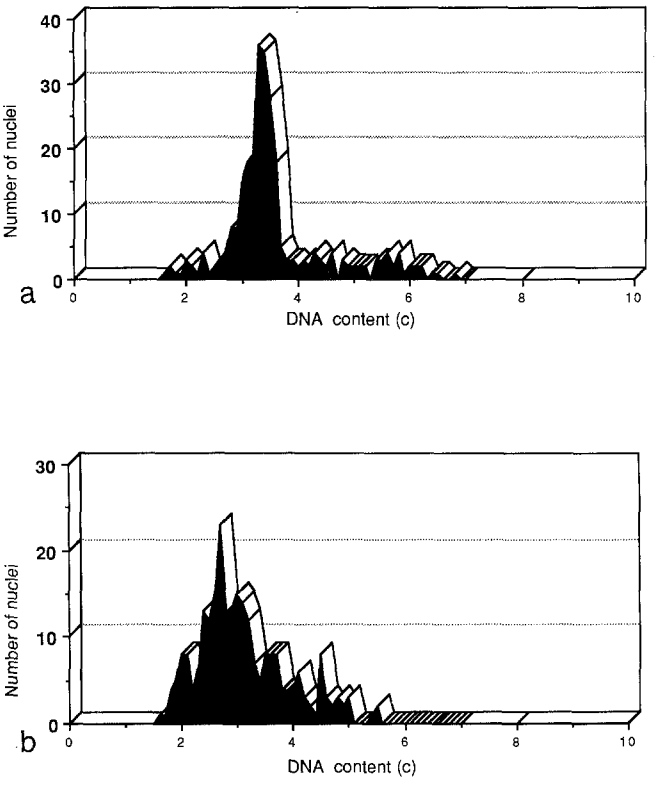

Fig. 2 Typical DNA histogram of **a** seminoma and **b** embryonal carcinoma

The Netherlands). This software identifies single nuclei and has the capability of artifact rejection. The histograms obtained are based on 320 objects identified as nuclei of epithelial morphology by the image analysis system. All objects are stored in a digitized memory buffer and in this study were consecutively controlled for over-looked artifacts by the user.

The resulting data are printed as DNA histograms. Using SAS-PC (SAS Institute, Cary, N.C.), several mathematical DNA indices of the histograms were calculated – mean ploidy, 5c exceeding rate, 2c deviation index, variation coefficient of the G0/1 fraction, DNA index, standard deviation of ploidy in G0/1 fraction, DNA malignancy grading and DNA nucleus diameter correlation [3, 4, 11, 19].

Results

Interpretation of DNA histograms

Of the 115 analyzed tumors, 109 were found to be aneuploid (95%). There was no significant difference in this between seminomas (98%), HCG-positive seminomas (92%) and embryonal carcinomas (92%). The tumors included 16.5% that characterized by multiclonality. The hypertriploid-to-hypotriploid ratio increased from HCG-positive Seminoma over embryonal carcinoma to seminoma (Fig. 1).

"Phenotypic" interpretation of the histograms revealed typical and different patterns for seminomas versus embryonal carcinomas. Seminomas are characterized by a distinct stem cell line in the peritriploid range in combination with S- and G2M-phase cells (Fig. 2a). Embryonal carcinomas, on the other hand, showed a heterogenous aneuploid DNA pattern without a clear-cut stem cell line (Fig. 2b).

DNA indices

The mean ploidy (MP) [19], the 5c exceeding rate (5cER) [3], the 2c deviation index (2cDI) [4], the variation coefficient of the G0/1 fraction (CV) [11] and the DNA nucleus diameter correlation (DNA/nucleus ϕ) discriminated with statistically significant correlation between the tumor types (Tables 2, 3). No significant difference could be calculated by the DNA index (DI) [11], the standard deviation of ploidy in G0/1 fraction (SDP) [11] or the DNA malignancy grading (DNA-Mg) [3].

Neither the phenotypic interpretation of the histograms nor the mathematical DNA indices showed any correlation with the pathological tumor stage of seminomas or non-seminomas.

Table 2 Definitions of mathematical DNA indices

MP:	mean value of sample/mean value of 2c population
5cER:	100× number of nuclei > 5c/number evaluated nuclei
2cDI:	1/number of evaluated nuclei × sum square of deviation of 2c
CV:	standard deviation G0/1 fraction/mean G0/1 fraction

Table 3 Significance level between the mathematical DNA indices

	Seminoma/HCG-pos. seminoma	Embryonal Ca/ seminoma
MP	$P=0.0229$	–
CV	–	$P=0.0001$
5cER	$P=0.0246$	$P=0.0244$
2cDI	$P=0.0029$	–
DNA/nucleus ϕ	–	$P=0.0073$

Discussion

In accordance with the available data in the literature [6–10, 15, 23], our results suggest that far more than 90% of the testicular germ cell tumors are characterized by non-diploid DNA patterns. However, for 6 of the 115 tumors analyzed in our series the DNA histograms were undoubtedly diploid. These have carefully been reviewed by our pathologist (D.P.) to exclude a wrong diagnosis of the primary tumor and/or the analysis of *non*-representative material for DNA cytometry. A diploid *DNA* histogram of testis tumors therefore does not exclude malignancy. Aneuploidy, on the other hand, is commonly regarded as a marker of malignancy. In a series of renal tumors, however, we found that oncocytomas, which always behave as benign neoplasms in our experience, were characterized by non-diploid histograms in a considerable percentage of cases [21].

Regarding the ability of the DNA cytometry to differentiate between various types of testicular tumors, the data in the literature are controversial. Some authors found no differences between seminomas and non-seminomas [8, 12], while others described the mean DNA index of seminomas as significantly higher than in nonseminomas [6, 7, 15]. We found a similar tendency in our material, but without statistical significance. Surprisingly, however, our marker-positive seminomas were also characterized by a low DNA index as well (embryonal carcinoma 1,37, HCG-positive seminoma 1,39, seminoma 1,45). The DNA-cytometric literature has so far not discriminated between marker-negative and marker-positive seminomas, probably because of the low frequency of the latter type.

Mathematical DNA indices have not formerly been calculated for testicular tumors. We found a significant correlation between some of these indices and the histological tumor type (Table 3). The differences between HCG-positive seminomas and marker-negative seminomas are again of special interest.

Some of the discrepancies between our data and those in the literature could be of methodological origin. The histograms obtained by image analysis DNA cytometry are almost exclusively based on the tumor cell population. Non-neoplastic cells and artifacts are excluded from DNA measurement by the image analysis system. Flowcytometric histograms, the basis of almost all the reports in the literature, are based on both neoplastic and non-neoplastic cell elements of the tumor.

A correlation between mathematical DNA indices and the prognosis of non-seminomas has so far been described by only one group [1] for the 5c exceeding rate. In our data, we found similar results [5].

Using a double-blind setting, we compared the DNA-histogram patterns of patients with clinical stage I disease, with reference to whether they had ($n = 33$) or did not have ($n = 41$) retroperitoneal dissemination during retroperitoneal lymphadenectomy.

The results demonstrated a DNA shift to a higher DNA content in advance of tumor growth. The Wilcoxon text revealed a significant increase (P 0.0165) in the percentage of nuclei with a DNA content of over 5c (5cER) in the group of patients with metastases. In addition, we compared the DNA histogram patterns of primary tumors with those of the corresponding lymph node metastases: we found a significant increase ($P = 0.005$) in the 5cER during the process of dissemination. DNA content of 2.25–4.5c and over 4.5c did not increase.

The results suggest that the 5cER can be regarded as a useful prognostic parameter in clinical stage I testicular cancer. We are currently reevaluating this correlation in a larger number of clinical stage I tumors. Confirmation would most probably indicate a high clinical impact of DNA cytometry in testicular cancer.

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References

1. Allhoff EP, Liedke S, Riese W de, Stief CG, Tanke HJ, Schneider B, Kramer AEJL, Jonas U (1991) Assessment of individual prognosis for patients with NSCGT/CS I. J Urol 145:367A
2. Atkin NB, Kay R (1979) Prognostic significance of modal DNA value and other factors in malignant tumours, based on 1465 cases. Br J Cancer 40:210
3. Böcking A (1982) Algorithmus für ein DNS-Malignitäts-Grading. Verh Dtsch Ges Pathol 66:540
4. Böcking A, Adler CP, Common HH, Hilgarth M, Granzen B, Auffermann W (1984) Algorithm for DNA-cytometric diagnosis and grading of malignancy. Anal Quant Cytol 6:1
5. Bürger RA, Braun MH, Witzsch U, Potratz D, Stöckle M, Hohenfellner R (1993) Automated DNA-image analysis in testicular cancer. Value of 5c exceeding rate as prognostic factor. J Urol 149:390A
6. Codesal J, Paniagua R, Regadera J, Fachal C, Nistal M (1991) Significance of DNA quantification in testicular germ cell tumors. Andrologia 23:381
7. El-Naggar AK, Ro JY, McLemore D, Ayala AG, Batsakis JG (1992) DNA ploidy in testicular germ cell neoplasms. Histogenetic and clinical implications. Am J Surg Pathol 16:611
8. Fosså SD, Pettersen EO, Thorud E, Melvik JE, Ous S (1985) DNA flow cytometry in human testicular cancer. Cancer Lett 28:55
9. Fosså SD, Nesland JM, Waehre H, Åmellem O, Pettersen EO (1991) DNA ploidy in the primary tumor from patients with non-seminomatous testicular germ cell tumors clinical stage I. Cancer 40:129
10. Graff WE de, Oosterhuis JW, Jong B de, Dam A, Putten WLJ van, Castedo SMMJ, Sleijfer DT, Schraffordt Koops H (1992) Ploidy of testicular carcinoma in situ. Lab Invest 66:166
11. Hiddemann W, Schumann J, Andreef M, Barlogie B, Hermann CJ, Leif RC, Mayall BH, Murphy RF, Sandberg AA (eds) (1984) Convention on Nomenclature for DNA Cytometry. Committee on Nomenclature, Society for Analytical Cytology. Cancer Genet Cytogenet 13:181
12. Kleinhans G, Hacker-Klom U, Göhde W, Körner F, Schumann J (1986) Zellkinetische Untersuchungen menschlicher maligner Hodentumoren. Urologe [A] 25:294
13. Koss LG, Wersto RP, Simmons DA, Deitch D, Herz F, Freed SZ (1989) Predictive value of DNA measurements in bladder washings. Comparison of flow cytometry, image cytophotometry, and cytology in patients with a past history of urethelial tumors. Cancer 64:916

14. Ljungberg B, Stenling R, Roos G (1986) Prognostic value of deoxyribonucleic acid content in metastatic renal cell carcinoma. J Urol 139:801
15. Oosterhuis JW, Castedo SMMJ, Jong B de, Cornelisse CJ, Dam A, Sleijfer DT, Koops HS (1989) Ploidy of primary germ cell tumors of the testis. Pathogenetic and clinical relevance. Lab Invest 60:14
16. Otto U, Baisch H, Huland H, Klöppel G (1984) Tumor cell deoxyribonucleic acid content and prognosis in human renal cell cancer. J Urol 132:237
17. Seppelt U, Sprenger E (1984) Nuclear DNA cytophotometry in prostate carcinoma. Cytometry 5:258
18. Sledge GW, Eble JN, Roth BJ, Wuhrman BP, Einhorn LH (1987) Flow cytometry derived DNA content of the primary lesions of advanced germ cell tumours. Int J Androl 10:115
19. Sprenger E, Volk L, Michaelis WE (1974) Die Aussagekraft der Zellkern-DNS-Bestimmung bei der Diagnostik des Prostatakarzinoms. Beitr Pathol 153:370
20. Stöckle M, Tanke HJ, Mesker WE, Ploem JS, Jonas U, Hohenfellner R (1987) Automated DNA-image cytometry: a prognostic tool in infiltrating bladder carcinoma. World J Urol 5:127
21. Stöckle M, Störkel S, Mielke R, Steinbach F, El-Damanhoury H, Voges G, Hohenfellner R (1991) Characterization of conservatively resected renal tumors using automated image analysis DNA cytometry. Cancer 68:1926
22. Winkler HZ, Rainwater LM, Myers RP, Farrow GM, Therneau TM, Zincke H, Lieber MM (1988) Stage D1 prostatic adenocarcinoma: significance of nuclear DNA ploidy patterns studied by flow cytometry. Mayo Clin Proc 63:103