Acta Clin Croat 2009; 48:201-206 Conference Paper

LECTURES

FEATURES OF PROSTATE NEEDLE BIOPSY CORES AS PROGNOSTIC FACTORS OF PSA RECURRENCE AFTER RADICAL PROSTATECTOMY

B. Spajić

University Department of Urology, Sestre milosrdnice University Hospital, Zagreb, Croatia

The aim of the study was to determine whether the percentage of carcinoma in needle biopsy and percentage of positive biopsy specimens as well as the localization of carcinoma in the prostate could be used as reliable predictors of biochemical relapse (BCR) in patients having undergone radical prostatectomy. The study included 340 patients that underwent radical prostatectomy at University Department of Urology, Sestre milosrdnice University Hospital, between 1996 and 2005. The mean follow up period was 50.3 months. New risk groups to assess BCR were based on the percentage of prostate carcinoma in total biopsy material and percentage of positive biopsy cores. They were matched with the existing risk groups based on preoperative prostate specific antigen levels (PSA) and Gleason score. Thus, the predictive value, time of onset and BCR-free survival rate in the newly formed groups were determined. In the newly formed groups, the percentage of carcinoma in needle biopsy and percentage of positive biopsy specimens were significant predictors of preoperative incidence risk and BCR-free survival ($P<0.0001$ both). Compared with the groups currently used for BCR-risk assessment, which are based on PSA and Gleason score values, the newly formed groups were superior in predicting BCR incidence risk and BCR-free survival ($P<0.0001$). Patients with bilobar prostate carcinoma showed a higher rate of BCR ($P=0.0002$) and shorter BCR-free periods compared to patients with unilobar carcinoma ($P<0.0001$). In conclusion, the newly formed risk groups based on the above mentioned histopathologic characteristics were useful in the assessment of BCR incidence and BCR-free survival. Thus, they could be used to recognize high-risk patients that may benefit from the introduction of adjuvant therapy. Besides, they could be used to assign primary therapy, i.e. to recognize patients in whom radiotherapy would yield similar results as prostatectomy. In such cases, radiotherapy is preferred as a less aggressive therapeutic option.

INTERPROFESSIONAL MEDICAL EDUCATION AND ROLE OF COMPARATIVE PATHOLOGY

S. Tkalčić

Western University of Health Sciences, Pomona, CA, USA

Interprofessional education is a fairly recent trend in medical education and practice with the goal to provide the most efficient and comprehensive approach to quality medical service and quality patient care. In this context, comparative pathology provides good support and common grounds to involve basic medical science in this process. Information explosion in medicine and biotechnology in the last few decades warrants the need for multiprofessional involvement and collaboration in medical diagnosis and patient care. Medical education is the first to introduce and start pilot programs to implement this trend into medical *curricula* worldwide. Small group settings with a Problem-Based Learning (PBL) approach are a good model for this effort. Many non-clinical and clinical competencies evolve from small group exercises in PBL, and in the same time, many learning issues generated from a given clinical scenario bring students from different backgrounds to contribute their expertise into collaborative efforts of case development. Different aspects of comparative pathology can bring students into the consideration of evidence based medicine in this process, by looking into mechanisms of disease, basic research using animal models, advances in pharmacology and clinical research, but also in consideration of public health and food safety issues in lieu of medical and veterinary pathology.

THE IMPACT OF 5-AZACYTIDINE ON CELL HOMEOSTASIS IN MAMMALIAN MALE GONAD

A. Katušić[1], E.G. Van Donselaar[2], A. J. Verkleij[2], F. Bulić-Jakuš[1], M. Vlahović[1], Lj. Šerman[1], N. Sinčić[1], F. Paić[1], G. Jurić-Lekić[3]

[1]Department of Biology, School of Medicine, University of Zagreb, Zagreb, [2] Cellular Architecture and Dynamics, Electron Microscopy and Structural Analysis, Faculty of Sciences, Utrecht University, The Netherlands, [3]Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia

The anticancer agent 5-azacytidine (5azaC, Vidaza) is a cytosine-based analog which causes DNA hypomethylation. As DNA methylation is an important mechanism of regulation of gene expression in gametogenesis, several studies have shown a severe, dose-dependent disruption of spermatogenesis in 5-azaC-treated adult animals. We investigated the effect of 5azaC treatment on fetal development of male gonad. 5azaC in a dose of 5 mg/kg was given to female Fisher rats by a single intraperitoneal injection on day 13, 14, 15 or 16 of pregnancy. On day 20, fetal testes were isolated and processed for routine histology or electron microscopy. Immunolabeling was done using two apoptotic markers, i.e. antibody against cleaved caspase-3 (Asp175) (Cell Signaling Technology) and antibody against early apoptotic marker γ-H2AX (Cell Signaling Technology), on semithin Tokuyasu cryosections. Light and electron microscopy revealed gonocytes in various stages of apoptosis in testes of all experimental groups. The highest incidence of apoptotic cells was detected in tubules of the group treated on day 15. However, less γ-H2AX positive cells were found, with the characteristic γ-H2AX foci in the nucleus and the early-to-middle apoptotic stage morphology. Testes of control fetuses were negative for the presence of apoptotic cells or any apoptotic marker. It was concluded that 5-azaC disturbed cell homeostasis in fetal rat gonad by causing apoptosis. Day 15 of pregnancy seemed to be the most sensitive period for the action of 5-azaC.

DNA METHYLATION STATUS DURING EXPERIMENTAL MOUSE TERATOCARCINOMA DEVELOPMENT

N. Sinčić[1], M. Vlahović[1], Z. Herceg[2], Lj. Šerman[1], F. Paić[1], A. Katušić[1], F. Bulić-Jakuš[1]

[1]Department of Medical Biology, School of Medicine, University of Zagreb, Zagreb, Croatia, [2]Epigenetics Group, International Agency for Research on Cancer, Lyon, France

Normal embryonal development and cancer development are regulated by the epigenetic mechanism of DNA methylation. Experimental mouse teratocarcinoma contains differentiated tissues and undifferentiated embryonal carcinoma (EC) cells. The aim of this study was to determine DNA methylation pattern of several genes possibly involved in teratocarcinoma development such as Brca1, Stat3, PRSS21 and SCGB3a1 and stemness genes Oct4, Sox2 and Nanog. In this experiment, 7.5-day-old C3H embryos were transplanted under the kidney capsule of syngeneic adults. Teratocarcinomas were isolated and weighed after 4 weeks and 8 weeks. DNA was isolated. After bisulfite conversion and PCR amplification, DNA methylation was analyzed by pyrosequencing. After 4 weeks, teratocarcinomas were much smaller than those obtained after 8 weeks. DNA methylation of transcription factor and tumor-suppressor genes was similar in the embryo and in both groups of teratocarcinomas, thus probably not being involved in embryonal cell transformation and growth of experimental teratocarcinoma. On the other hand, stemness genes were less methylated in the embryo than in teratocarcinoma. We can speculate that DNA methylation reprogramming of the mentioned genes during cancer development is due to cell differentiation of the teratoma component within teratocarcinoma. Further intensive growth of teratocarcinoma after 4 weeks is probably the consequence of established stability of DNA methylation statuses between undifferentiated EC component and already differentiated cells allowing EC cells intensive proliferation without differentiation.

ANTIOXIDANT PBN AMELIORATES THE TERATOGENIC EFFECT OF 5-AZACYTIDINE IN RAT

N. Sobočan[1], N. Sinčić[1], A. Katušić[1], Lj. Šerman[1], T. Nikuševa-Martić[1], G. Jurić-Lekić[2], F. Paić[1], M. Vlahović[1], F. Bulić-Jakuš[1]

[1]Department of Biology, [2]Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia

The effect of the antioxidant N-tert-butyl-á-phenylnitron (PBN) on teratogenesis induced by the DNA demethylating agent 5-azacytidine (5azaC) was investigated in rat. On days 12 and 13 of gestation, Fisher rats were pretreated by an i.v. injection of PBN (40 mg/kg) and one hour later treated by an i.p. injection of 5-azaC (5 mg/kg). On days 15 and 20 of gestation, fetuses were isolated and their development compared. PBN treated fetuses developed with no difference from untreated controls. PBN significantly improved survival in combination with 5azaC, although this survival was still significantly worse than in controls. On day 15 of gestation, embryo weight was similar in controls, PBN and PBN/5azaC treated embryos, whereas 5azaC treated embryos were significantly smaller. On day 20, embryos were significantly smaller also in the PBN/5azaC treated group, although their weight was significantly higher than in the 5azaC treated group. Similar results were also obtained for crown-rump lengths, which were longer after PBN pretreatment on both days 15 and 20. Limb malformations were found in all fetuses treated with 5azaC, even in those pretreated with PBN. However, after PBN pretreatment, the number of adactyly in front- and hind-limbs was significantly smaller and of oligodactyly significantly higher as compared with only 5azaC treated group. In limb-buds, cell proliferation (Nv PCNA) was significantly lower in 5azaC treated group than in controls and slightly higher in PBN pretreated group. PBN significantly improved most of developmental parameters when used as a pretreatment to 5azaC, which can lead to a conclusion that the teratogenic impact of 5azaC could at least partially depend on the activation of oxidative stress pathways.