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Thesis Abstracts

CYTOGENETIC STUDY OF BENIGN AND MALIGNANT HUMAN NEOPLASIAS

Nilce Barril*

The present study investigated factors influencing *in vitro* cell proliferation, and clonal and nonclonal chromosome abnormalities in short-term cultures initiated after collagenase disaggregation of biopsies from 18 solid tumors.

The analyses of culture conditions were performed with three benign and six malignant tumors. The results showed that the factors inducing good cellular growth of specific cells were the plating of 2.02×10^4 cells/ml in Descarplast or Leighton flasks with Ham F-10 medium supplemented with 20% fetal calf serum, glutamine, insulin and estrogen.

The cytogenetic studies were performed on seven benign and four malignant tumors. They were three inflammatory lesions and one carcinoma *in situ* of the uterine cervix, one stomach adenocarcinoma, four multinodular goiters of the thyroid, one synovial sarcoma and one cystic carcinoma of the salivary gland.

Two inflammatory lesions of the uterine cervix showed normal karyotypes in very few cells, and two carcinomas and three goiters showed nonclonal aberrations, mostly numerical deviations, in particular, chromosome losses. Besides normal and hypodiploid metaphases, one variant cell with an abnormal constitution, 92,XXXX, del(4)(q22), was seen in one inflammatory lesion of the uterine cervix. The abnormality was relevant with regard to observations in earlier studies which indicated i(4p) or i(p5) as one of the most common aberrations in carcinomas of the uterine cervix.

Cytogenetic analysis of one synovial sarcoma revealed chromosome numbers of less than 46. This finding may be correlated with advanced stages of the disease as seen in ovarian and breast tumors.

Three clones were detected in a multinodular goiter, 47,XX,+7, 48,XX,+7,+17 and 49,XX,+7,+10+17. Trisomies 7, 10 and 17 are common findings in hematologic malignancies

and in solid tumors and different genes located on those chromosomes are related to cell proliferation or thyroid function. However, trisomies 7 and 10 have been reported in short-term cultures of nonneoplastic cells, demonstrating genomic instability in the tumor parenchyma, but they may also characterize stromal fibroblasts.

Finally, one abnormal clone was found in one adenoid cystic carcinoma of the salivary gland with karyotype 45,XY,-22, del(10)(p12). Deletion of 10p is found in a wide variety of leukemias and lymphomas and monosomy 22 is often present in patients with neurogenic tumors. However, neither has been seen in tumors of the salivary gland. Clinical and histological details and data of previous cytogenetic studies suggest that the present case may represent an ameloblastoma and not a tumor of the salivary gland. The results emphasize that cytogenetic analysis may provide valuable diagnosis and also prognostic information of benefit to patient management.

* 1991. Departamento de Biologia, IBILCE-UNESP, São José do Rio Preto, SP, Brasil. Master's Thesis. Orienting Professor: Eloiza Helena Tajara da Silva.

ULTRASTRUCTURAL STUDY OF SPERMATOGENESIS AND OF SPERM OF *Cosmopolites sordidus* GERMAR (COLEOPTERA: CURCULIONIDAE)

José Lino Neto*

The spermiogenesis of *Cosmopolites sordidus* was investigated with the use of thin sections, negative staining preparations, and scanning electron microscopy of spermatid cells obtained from the testicles or spermathecae. In this species, as with insects in general, this process is characterized by the following events: flagellar formation, chromatin condensation, nuclear elongation and shedding of excess cytoplasm. Early spermatids are similar to somatic

cells in that they have spherical nuclei with predominantly loose chromatin, a typical Golgi complex and many scattered mitochondria. These mitochondria fuse to form a mitochondrial complex made up of two distinct mitochondrial structures which curl around each other. In the first stages of spermatogenesis, chromatin begins to condense at the nuclear periphery and from this dense mass, regular filaments are formed. These filaments fuse into thick cords which, in turn, associate laterally, resulting in semi-compact chromatin with hexagonally arranged clear spaces. Finally, with the disappearance of these spaces, a homogeneously compact nucleus is obtained, typical of spermatozoa. The nuclear shape changes, simultaneously with chromatin condensation, from spherical to cylindrical, and is surrounded by a single layer of microtubules. The proacrosomic vesicle already appears at early stages of spermiogenesis, through Golgi vesicle fusion and develops into a triple structure, consisting of the perforatorium, the acrosomic vesicle and a layer of extra-acrosomic material. The mitochondrial complex divides into two elongated mitochondrial derivatives. In the spermatozoa, these derivatives are not equal; the larger reaches twice the diameter of the smaller and is almost totally filled with material in a paracrystalline arrangement. The smaller derivative is mostly taken up by regularly spaced cristae, arranged perpendicularly to its greater axis. The flagellar axoneme originates from a single centriole observed in the early spermatid. The axoneme, typical for insects, consists of two central microtubules surrounded by nine doublets, from which nine accessory microtubules are later formed. During nuclear elongation, the centriolar adjunct appears, consisting of an electron dense ring, which surrounds the centriole. This centriolar adjunct disappears before spermiogenesis is completed. In later stages, the accessory bodies are formed on each side of the axoneme. In the mature flagellum, these structures, observed in transverse section, are triangular and partially surround the axoneme. Each dense, accessory body has a less compact extension which is considerably larger for one of them. During spermatid differentiation, most of the cytoplasm, together with the microtubules, Golgi complex, endoplasmic reticulum and free polisomes are eliminated, resulting in a long, thin spermatozoon, about 160 μm in length.

THE EFFECT OF *Maytenus illicifolia* MART. EX. REISS AND *Achillea millefolium* (YARROW) ON THE SPERMATOGENESIS OF SWISS ALBINO MICE

Tatiana Montanari*

The effects of *Meytenus illicifolia* Mart. ex. Reiss and *Achillea millefolium* (yarrow) on the spermatogenesis of Swiss albino mice was tested, evaluating morphological characteristics with the light and electron microscopes. These species are medicinal plants which have been used in popular medicine for various cures and as fertility regulators.

A daily dose of 200 mg/kg of the alcoholic extract of *M. illicifolia* leaves was administered intraperitoneally, for 20 days, and a daily dose of 800 mg/kg, orally, for 30 days. The alcoholic extract of *A. millefolium* flowers was administered daily at doses of 200 mg/kg, intraperitoneally, for 20 days and 300 mg/kg of the hydroalcoholic extract, orally, for 30 days.

The alterations observed in testes treated with *M. illicifolia* and *A. millefolium* included immature germ cells, germ cell necrosis (recognized as picnotic and hypertrophic nuclei) and vacuolized seminiferous tubules. These variations were not the same in the different experiments. In some controls, picnotic nuclei and some vacuolized tubules were also found.

Cell structure of animals treated with 200 mg/kg/day or 800 mg/kg/day of *M. illicifolia* was not noticeably different from that of controls. Ultrastructurally, greatly enlarged lipid droplets were found in Sertoli cells and dilated, deformed acrosomes occurred in early spermatids of animals treated with the higher dose. Normal spermatogenesis was found in most of the seminiferous tubules of mice treated with *M. illicifolia*. Sperm production indicated that its alcoholic extract did not contain sufficient substances to arrest the spermatogenic process.

Seminiferous tubules of animals treated intraperitoneally with *A. millefolium* at a dose of 200 mg/kg/day for 20 days were the most severely affected. Many tubules were exfoliated, with immature germ cells occupying the lumen and with reduction of the germ cell layer. Animals treated with 300 mg/kg/day of *A. millefolium* had tubules with an increased number of metaphases, which may be a consequence of cell cycle blockage or be due to a proliferation stimulus, caused by the plant extract. The fact that the testes of intraperitoneally-treated animals were affected suggests that the active principle has a low

* 1993. Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil. Master's Thesis. Orienting Professor: Mary Anne Heidi Dolder.

concentration in the hydroalcoholic extract or that it was degraded during oral absorption.

* 1994. Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil. Master's Thesis. Orienting Professor: Mary Anne Heidi Dolder.

CYTOGENETIC STUDY OF BENIGN LESIONS OF THYROID AND SALIVARY GLANDS

*Patrícia de F. Faleiros-Ribeiro.**

Several human neoplasias are specifically related to particular chromosome aberrations. These aberrations determine activation or suppression in gene functions, which can be involved in neoplastic initiation or progression. A search was made for chromosome abnormalities in cells derived from thyroid and salivary gland proliferative lesions. Cytogenetic evaluation was made of short-term cell cultures from five thyroid colloidal multinodular goiters and one pleomorphic adenoma specimen.

A clonal abnormality, $t(3;8)(p21;q12ou13)inv(8)(p12q11)$ was found eighteen cells of the parotid pleomorphic adenoma. This patient had a previous history of four surgeries for tumor resections in this same site.

Four patients with thyroid colloidal multinodular goiter expressed only non-clonal chromosome gain, losses and rearrangements. However, the fifth patient, besides displaying aneuploidies also presented a clonal complex chromosome translocation in all the cells analyzed. Four chromosomes (#1, #4, #9 and #13) were involved in an apparently unbalanced rearrangement. The occurrence of a complex rearrangement in a benign non-neoplastic lesion raises questions both on the accuracy of the diagnosis of the lesion and on the association between this cytogenetic event and the cell transformation process. These findings confirm the importance of the cytogenetic evaluation in benign proliferative lesions.

A STUDY OF FETAL KARYOTYPE IN UMBILICAL BLOOD SAMPLES OBTAINED BY CORDOCENTESIS AFTER THE 19th WEEK OF GESTATION

*Maria Elisa Sportello**

We performed cytogenetic analyses on 254 fetal blood samples obtained by cordocentesis from 252 pregnant women (including two twin pregnancies) with gestational age ranging from the 19th week to full term, between september/1988 and february/1994.

This study had the following objectives:

1. Investigation of numerical and structural chromosome abnormalities in fetal blood samples.
2. Establishment of the frequency of these abnormalities in the group indicated by fetal malformation detected through ultrasonography and compare it with the other indications.

The main indications for cytogenetic fetal analysis were: detection of fetal malformation by ultrasonography (74.4%), Rh isoimmunization (17.5%), maternal infection and others (8.1%).

The fetal karyotype was obtained in 223 samples. The failures were due to maternal blood contamination, absence of cellular growth, bacterial contamination of the samples that were associated with maternal infection, individual cellular variation and sampling accidents. We found 21 chromosome aberrations, all of them in the fetal malformation group.

The chromosome aberrations found were: 47,XX,+13 (one case), 47,XY,+13 (one case), 47,XX,+18 (five cases), 47,XY,+18 (five cases), 47,XX,+21 (one case), 47,XY,+21 (one case), 45,X (one case), 46,XY,del(13)(q22.3) (one case), 69,XXX (one case), 46,XY/47,XY,+9 (one case), 45,X/46,XX (two cases) and 46,XX/46,XX,+t(13q14q) (one case).

We observed an association between chromosome aberrations and anomalies of the main organs and systems, and its rate increased when there were multiple abnormal events, intrauterine growth retardation or alteration of amniotic fluid volume. Similar observations were related in the literature.

C heteromorphisms were observed in 5% of the cases, especially involving heterochromatin inversion of 9q, which is common finding in the general population.

* 1994. Departamento de Morfologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, SP, Brasil. Master's Thesis. Orienting Professor: Leila M.S. Farah.

* 1994. Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, IBILCE-UNESP, São José do Rio Preto, SP, Brasil. Master's Thesis. Orienting Professor: Marileila Varella-Garcia.