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Review

Of Mice and Men: Teratomas and Teratocarcinomas

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A B S T R A C T

Teratomas and teratocarcinomas are tumors containing tissue derivatives of all three germ-layers. They can be induced by transplantation of animal embryos to ectopic microenvironment. Development of malignant teratocarcinomas depends on embryonic stage, species-specificity and immunological competence of the host. In the man, teratomas and teratocarcinomas usually represent a subtype of germ-cell tumors but sacrococcygeal teratomas arise from the remnants of the pluripotent primitive streak. Undifferentiated embryonal carcinoma (EC) cells are responsible for the malignancy of experimental mouse teratocarcinomas. Mouse EC cells injected to the adult give rise to tumors and upon injection to early embryos to differentiated tissues – thus resembling normal mouse embryonic stem cells (mESC). Epigenetic changes rather than mutations are associated with transformation of mESC to EC cells. Human EC and ES cell-lines (hESC) contain chromosomal abnormalities and can form teratocarcinoma after transplantation. ES cells are among those proposed for cell replacement therapy in the man. Suicide gene introduction should be recommended prior to their use in vivo to ablate them in case of malignant transformation.

***Key words**: teratoma, teratocarcinoma, embryo, EC cell, ES cell, cell therapy*

Experimental Animal Teratoma and Teratocarcinoma

Experimental teratomas, tumors containing a disorganized mixture of various tissues, were induced in laboratory animals such as rat by subcapsular kidney transplantation of postimplantation embryos or their parts. In such a favorable *in vivo* microenvironment well differentiated derivatives of the ectoderm, mesoderm and endoderm (the three germ-layers) such as brain tissue, epidermis, bone, muscle and gut epithelium regularly differentiated. Tissues were sometimes even forming organotypic structures resembling e.g. the tooth, fingers with phalange etc. Švajger, Levak-Švajger and Škreb have discovered that, depending upon the stage of embryonic development, single germ-layers differ in their potential to give rise to differentiated tissues. Embryonic epiblast (pre-gastrulation primary ectoderm) formed a teratoma containing differentiated tissues derivatives of all three definite germ-layers. Primary endoderm (hypo- blast) isolated at this stage was resorbed. After the formation of the primitive streak, embryonic ectoderm was able to give rise to ectodermal and mesodermal derivatives but not of the endodermal so that a restriction of its developmental potential occurred in older embryos. The general conclusion, later corroborated by experiments of others, was that all three definitive germ-layers in mammals are originating from the primitive ectoderm (epiblast)[1,2].

Postimplantation rat embryos (E9, 5) cultivated for two weeks *in vitro* also gave rise to experimental teratomas. Surprisingly, their developmental potential was partly executed even in the protein-free chemically defined medium[3]. In this medium, targeted changes of differentiation were achieved by addition of defined growth, differentiation or morphogenetic factors (e.g. transferrin promoted differentiation of the ocular lens cells and RA

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changed differentiation from epidermis to columnar epithelium)[4–6]. It was also noticed that the restriction of developmental potential for neural tissue differentiation found after serumless *in vitro* culture itself was retained in transplants in spite of the fact that this microenvironment is very favorable for differentiation of directly transplanted embryos[7,8].

Solter, Škreb and Damjanov have found that by transplantation of the mouse egg-cylinder under the kidney capsule, not only teratomas, but also retransplantable teratocarcinomas could develop. Among differentiated tissues, teratocarcinomas contained undifferentiated embryonal carcinoma cells (EC)[9]. Older embryos going through the process of neurulation were not able to give rise to teratocarcinoma, probably because ectoderm was already developmentally committed and has lost its pluripotentiality[10,11]. Development of tumors occurred also in testicular transplants, but their weight was significantly lower. Embryo-derived tumors caused splenomegaly in hosts which was greater in animals bearing teratocarcinoma[12]. Interestingly, experimental teratocarcinoma was not found in rat transplants, but sometimes yolk-sac carcinoma developed. Yolk-sac carcinoma developed also in the mouse but after a longer period of time[10]. Teratocarcinoma development was dependent on the mouse strain. However, strains that did not permit teratocarcinoma development did so in F1 hybrid hosts[11]. In immunologically compromised mice, teratocarcinoma development was rare[13]. From the above described experi- ments it can be concluded that development of malignant teratocarcinomas depends on embryonic stage, species- specificity and immunological competence of the host. Although genetic factors seem to be important for the rise of embryo-derived teratocarcinoma, main culprits for the development of this malignant tumor are seemingly epigenetic factors from the microenvironment at the ectopic site which change gene expression and potential for differentiation of transplanted embryos[14].

Human Teratoma and Teratocarcinoma

Human germ cell tumors (GCT) can be either testicular or ovarian. They may share important etiological factors but incidence of female GCT is much lower[15,16]. The research of their cause has been guided by the hypothesis that the disease process starts in fetal life with the abnormal differentiation of fetal primordial germ cells. Testicular germ cell tumors are divided into two groups: seminomas and nonseminomatous germ cell tumors (NSGCT). Intratubular germ cell neoplasia of unclassified type (IGCNU) seem to be a precursor for those lesions[15]. NSGCT[16] are thought to have a clonal origin and to recapitulate embryogenesis, their pattern of differentiation being directed toward the formation of one or more of the components of the embryo and related structures. The specific direction this differentiation takes will determine the morphologic appearance of given tumor and hence its name[15,17]. There are four basic patterns of NSGCT: embryonal carcinoma (primitive carcinoma like cells with minimal or without signs of differentiation), mature and immature teratoma (differentiation toward structures of the embryo proper), choriocarcinoma (presence of well-developed trophoblastic elements in an organoid fashion), yolk sac tumor (formation of extraembryonic endoderm and mesoderm). Tumors exhibiting two or more of these patterns are designated as mixed NSGCT. The combination of embryonal carcinoma and teratoma is also known as teratocarcinoma. Current morphologic, cytogenetic and DNA ploidy data are showing that seminoma probably serves as precursor in the formation of NSGCT[17,18].

A cryptorchid testis is 30–50 times more likely to develop a malignant neoplasm than a normally placed organ. The incidence of testicular cancer is also increased in men with hypospadias and with inguinal hernia. There are some tumors that have occurred in a family setting, suggesting a genetic background but also environmental exposures to pesticides, textile dust, organ solvents seem to be important[15,19].

The majority of testicular germ cell tumors manifest aneuploid DNA contents with minimal intratumoral heterogeneity. Seminoma and IGCNU cells are hypertriploid and NSGCT are hypotriploid. They have at least one X and one Y chromosome. Mature teratoma of the prepubertal testis is the only TGCT lacking gross chromosomal aberrations. TGCT of all other types are characterized with two abnormalities of chromosome 12[20], |i(12p)| in about 80% and |del(12q)| in 20%. It has been postulated that these deletions cause the loss of one or more tumor suppressor genes whose products regulate the normal proliferation of spermatogonial germ cells[20,21]. |i(12p)| is also detected in these types of tumors in ovary, mediastinum and midline of the brain. Persons with 46, XY or 45, X/46, XY are at very high risk of gonadal germ cell tumor. Telomerase activity is present in all types of TGCT which can be explained with biallelic expression of multiple imprinted genes. Cyclin E has a higher expression in embryonal carcinoma than in other NGCT and Fas gene mutations are also common in that tumor[15]. Teratocarcinoma were found to be hypermethylated while seminomas were hypomethylated which epigenetic difference might reflect the normal developmental switch in primordial germ cells from an undermethylated genome to a normally methylated genome[22,23].

Sacrococcygeal teratoma (SCT) is developing at the caudal end of the primitive streak (a transient formation of the gastrulating embryo containing pluripotent cells), probably from its remnants which did not disappear on time[24]. It is predominantly benign and can contain all kinds of differentiated tissues among which even cells of the ocular lens[25]. Sacrococcygeal teratoma expresses several oncoproteins and tumor suppressor proteins such as ras, fos and jun, nm23 and p53 but no correlation was found between intensity of their expression and tumor size, age and survival of patients neither between mature and immature type of tumor[26].

EC and ES Cells

EC cells were isolated from experimental animal teratocarcinomas and human teratocarcinomas and subjec-

ted to extensive investigation from 1954 until today. In the mouse, introduction of a single EC cell to the blastocyst was able to produce normal chimeric mice, while in adults subcutaneous or intraperitoneal introduction of EC cells produced teratocarcinoma[14,27–29]. Adult chimeras, or later stages of postimplantation embryos especially after completion of organogenesis[30], also develop tumors and it seems that the microenvironment of the early embryos is more favorable for the suppression of malignant phenotype. This shows that the development of the teratocarcinoma is epigenetically regulated by the microenvironment and not caused by mutations which are found in majority of other types of tumors[14].

Because pluripotentiality of EC cells resembles pluripotentiality of ES cells – cells of the inner cell mass from the blastocyst which gives rise to the embryo proper, EC cell lines were established *in vitro* to investigate biologically active molecules in mouse development. Later on, establishment of pluripotent mouse embryonic stem cells (mESC) in cultures *in vitro* made possible the production of transgenic gene knock-outs in mice. Pluripotent ES rat cells could not be cultivated[28]. So in the rat, two species-specific differences in comparison to the mouse were found, namely no teratocarcinoma in embryonic transplants and no pluripotent ES cell-lines *in vitro*. Mouse embryonic stem cells (mESC) could also induce subcutaneous tumors, but they were growing at a much slower rate in direct comparison to teratocarcinoma derived from transplantation of an euploid EC cell-line. Gene expression profiling on microarrays was done to investigate differences in gene expression between teratocarcinoma and ES control in both cell cultures and in nude mice tumors. Results have shown the involvement of several pathways, and especially the cell cycle pathway in induction of teratocarcinoma[31].

Human EC cell-lines were established from human germ cell primary or metastatic tumors both *in vivo* and *in vitro*. Human EC cell-lines are virtually always aneuploid and only few can differentiate into well recognizable cell types. In both males and females extra-gonadal germ-cell tumors are usually diploid and very few cell-lines have been developed from them[27]. Specific biological differences between animal and human EC cells include a distinct pattern of surface antigen expression[32].

Human embryonic stem cells (hESC) were recently cultivated *in vitro* and cell lines were established. They seem ready to develop chromosomal abnormalities in long-term *in vitro* cultures among which i(12p), strongly implicated in human germ cell cancer[33]. It was also reported that long-term cultivated hESC can induce teratocarcinoma after transplantation to immunodeficient SCID mice[34].

Cell Replacement Therapy

The latest rise of hopes in regenerative medicine based on cell replacement therapy, tissue or organ engineering[35,36] are in fact funded upon developmental biology research aimed to investigate potential for growth and differentiation of various immature cells in an embryo, teratocarcinoma and in an adult organism[29]. Cells that are today in focus for therapeutic purposes are pluripotent embryonic stem cells (ES) from the blastocyst obtained either from the surplus of embryos after *in vitro* fertilization or possibly after therapeutic cloning. Of wide developmental potential are also PGE (primordial germ cells), cord blood stem cells or stem cells isolated from the adult organism which are not derived from sources that are subjected to wide ethical discussions as the ones previously mentioned[35,36].

Cell replacement therapy can be exerted through direct approach by transplanting cells directly from one organism to the other, or even to an embryo[37]. Fetal human mesenchymal stem cells from the liver of un unrelated donor were shown to alleviate a case of osteogenesis imperfecta (a disease characterized with multiple prenatal and postnatal bone fractures) in a three-year-old child subjected to therapy *in utero*[38]. Recently a therapeutic success was reported with the human bladder in several patients. Muscle and urothelial cells were taken from the miniature bladders of the patients themselves and propagated *in vitro* upon a degradable scaffold. Thus a whole organ was constructed and successfully transplanted back to the patient[35].

Although a lot is known about the biology of various kinds of undifferentiated cells, in order not to compromise the therapeutic effect with an aberrant development resulting with tumors, basic research is still necessary. In fact, one case of the tumor development was reported in a cell therapy experiment in the mouse. Mouse ES cells were differentiated into neural cells *in vitro* and subsequently transplanted subretinally. After two months, a teratoma appeared making the whole eye nonfunctional. Probably the *in vitro* differentiation process was not completed in every ES cell and some remained undifferentiated producing a tumor after transplantation[39]. In the latest fuctional engraftment of human ES cell-derived dopaminergic neurons to the parkinsonian rats, potential for phenotypic instability and undifferentiated expansion was reported[40]. Another danger lies in usage of immunocompromized mice for testing the developmental potential in transplants of various undifferentiated human cells because it was shown that in immunologically compromised mice, teratocarcinoma development from transplanted embryos was rare[10]. If immunologically compromised mice are also not able to readily produce tumors from undifferentiated human cells, then these tests are not totally reliable. Therefore, research involving the transduction of suicide genes to mouse or human stem cells seems to be especially important because such genes could render stem cells prone to ablation on demand and make a »fail-safe protection against cellular misbehavior«[41,42].

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O MIŠEVIMA I LJUDIMA – TERATOM I TERATOKARCINOM

SAŽETAK

Teratomi i teratokarcinomi su tumori koji se sastoje od tkivnih derivata svih triju zametnih listića. Moguće ih je inducirati transplantacijom animalnih zametaka u ektopični mikrookoliš. Razvoj malignog teratokarcinoma ovisi o stadiju razvoja zametka, species-specifičnosti te imunološkoj kompetenciji domaćina. U čovjeka, teratomi i teratokarcinomi obično predstavljaju podtipove tumora spolnih stanica, ali sacrococcygealni teratom nastaje iz zaostataka pluripotentne primitivne pruge. Nediferencirane stanice embrionalnog karcinoma (EC) odgovorne su za malignost eksperimentalnog mišjeg teratokarcinoma. Mišje EC stanice injicirane odraslom stvaraju tumore, a injicirane u rane zametke diferencirana tkiva te stoga nalikuju normalnim mišjim matičnim stanicama zametka (mESC). Epigenetske promjene, prije nego li mutacije, povezane su s transformacijom mESC u EC stanice. Ljudske EC i ES stanične linije (hESC) sadrže kromosomske aberacije i mogu formirati teratokarcinom nakon transplantacije. ES stanice su među stanicama predloženim za staničnu nadomjesnu terapiju čovjeka. Trebalo bi preporučiti da se u njih unesu samoubilački geni prije upotrebe *in vivo*, kako bi se mogle odstraniti u slučaju maligne transformacije.