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MINIREVIEW

Tumor Stem Cells

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Stem cells possess two basic characteristics: they are able to renew themselves and to develop into different cell types. The link between normal stem cells and tumor cells could be examined in three aspects: what are the differences and similarities in the control of self-renewal capacity between stem cells and tumor cells; whether tumor cells arise from stem cells; do tumorous stem cells exist? Since tumor cells also exhibit self-renewal capacity, it seems plausible that their regulation is similar to that of the stem cells. The infinite self-renewal ability (immortalization) is assured by several, so far only partly known, mechanisms. One of these is telomerase activity, another important regulatory step for survival is the inhibition of apoptosis. Other signal transduction pathways in stem cell regulation may also play certain roles in carcinogenesis: e.g. Notch, Sonic hedgehog (SHH), and Wnt signals. Existence of tumor stem cells was suggested since it is simpler to retain the self-renewal capacity than to reactivate the immortality program in an already differentiated cell. Moreover, stem cells live much longer than the differentiated ones, and so they are exposed for a long period of time to impairments, collecting gene errors leading to the breakdown of the regulation. However, it is still an open question whether all cells in the tumor possess the capacity that produces this tissue or not, that is: are there tumor stem cells or there are not. If tumor stem cells exist, they would be the main target for therapy: only these must be killed since the other tumor cells possess limited proliferative capacity, therefore limited life span. The only problem is that during tumor progression stem-like cells can develop continuously and the identification but mainly the prevention of their formation is still a great challenge. (Pathology Oncology Research Vol 10, No 2, 69–73)

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Stem cells and tumor cells

Fetal stem cells isolated from human fetal tissues are able to form various differentiated cell types originated from all three germ layers.[1] This discovery led to the idea of tissue or organ production with all its ethical problems. It was a milestone when the plasticity of somatic or adult stem cells in already differentiated tissues was proved that they not only produce one particular cell-line but also cell types that have not occurred in the original tissue of that given stem cell. This transdifferentiation (e.g. liver cell, kidney cell, myocardial cell, nerve cell developing from bone marrow stem cell)[2-7] occurs in a different microenvironment where a genetic program is activated that does not function in the place of origin. The new environment means primarily growth and/or differentiation factors, partly produced by the cells (like stroma cells) in the new place, partly located in the extracellular matrix. The effects of these factors will define the program that will drive the stem cell towards proliferation and differentiation.[8]

Stem cells possess two basic characteristics: they are able to renew themselves and to develop into different cell types. The capability for differentiation may be *totipotent* (the fertilized oocyte, the zygote develops the embryo and the trophoblasts of the placenta), *pluripotent* (almost all cell types of the germ layer), *multipotent* (more cell types on a certain locations) and *unipotent* (single cell type). These abilities may result in, through an asymmetric cell division, a stem cell and a cell committed to differentiation maintaining the balance of that particular tissue. (The formation of two stem cells or two differentiated cells is also conceivable.) Stem cells exist probably in all organs

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with the possible exception of the heart. Stem cells represent 1-2% of all the cells in most tissues.

In connection with tumors the issue of stem cells has been raised for several decades. It was found that

- stem cells and tumor cells have common features concerning their unlimited proliferative and tissue-specific differentiation capacity;
- the clonogenic potential, the capacity for self-renewal and differentiation is characteristic only for a certain population of tumor cells;
- in some tumor cells terminal differentiation, i.e. loss of proliferative ability was triggered by natural differentiation factors or exogenous compounds.[9]

In the 1970s the assays using mostly normal hemopoetic stem cell methodology represented the so-called clonogenic assay (stem cell assay) – *in vitro*: e.g. agar-colony test, *in vivo:* spleen colony test, lung colony test - examining what percentage of the tumor cells can form a new tumor colony.

These methods later were used in metastasis research since the tumor cells given i.v. generated lung metastasis (artificial metastasis) indicating the colony forming and metastasizing capacity alike. The relation between these two phenomena is apparently assumable but the experiments got stuck at this stage.

It would go too far to analyse the similarities between stem cells and metastatic cells. The key issue in both cases is determining the role of the microenvironment considering, for example, the problems of the organ-specific metastatization.

Observations that manipulation of the tumor cells or the target organ by changing the environment (by radiation, immunosuppression, cytotoxic injury, etc.) the clonogenic/metastasizing ability can be modified made the clonogenic/metastatic studies particularly vulnerable. In spite of the numerous in vitro and in vivo experiments the presence of the stem cells in the tumors is still an open question.

The link between normal stem cells and tumor cells could be examined in three aspects:

- what are the differences and similarities in the control of self-renewal capacity between stem cells and tumor cells;
- whether tumor cells arise from stem cells;
- do tumorous stem cells exist?

Regulation of self-renewal capacity

Stem cells will be renewed in a regulated manner and give source to cell differentiation, providing the amount of cells necessary for a given tissue structure. Nevertheless, stem cells are not exact copies of each other.

Several stem cell types exist in the bone marrow (long-life hemopoetic stem cells, short-life hemopoetic stem cells, multipotent progenitor cells) that are responsible for forming less and less cell types in a staggered way, and as their names indicate, their life span is also different.

Although we know quite well the phenotypes of the different stem cells, we know very little about their regulations. Since tumor cells also exhibit self-renewal capacity, it seems plausible that their regulation is similar to that of the stem cells. The infinite self-renewal ability (immortalization) is assured by several, so far only partly known, mechanisms. One of these is telomerase activity that inhibits the shortening of chromosomes in the course of multiplication and cell death when reaching the critical length. Such or similar mechanisms are crucial for stem cells and can play an important role in tumor formation. Indeed a significant number of tumors show high telomerase activity. (This of course is generalization, since – although very rarely – it might occur that tumor cells do not grow but spontaneously regress. Although, in this last case the reason might be quite different e.g. insufficient blood supply.) The other important regulatory step for survival is the inhibition of apoptosis especially in cell systems where the cells die without survival factors (like in the lymphoid system). Bcl-2 overproduction is an example of what has been observed in several – among them many solid – tumors. (First, it has been observed in follicular lymphomas, as a result of a t:14,18 translocation.) It turned out that high Bcl-2 expression may increase the number of hematopoietic stem cells.[10,11] By now it has become obvious that different antiapoptotic strategies can be effective in different celltypes, and the members of Bcl-2 family are only one set of players. At the regulatory level the inhibition of apoptosis is closely related to survival programs (e.g. Akt expression). Overexpression of such survival factors can induce immortality, even when the given cell loses this capacity under normal circumstances. No doubt, survival factors are needed to maintain stem cells.

Other signal transduction pathways in stem cell regulation may also play certain roles in carcinogenesis: e.g. Notch, Sonic hedgehog (SHH), and Wnt signals.[12-15] It is suggested that the mutations of these pathways support malignant transformation. The significance of such signals in the renewal of hematopoietic stem cells is widely accepted, and data are accumulated on their similar role in the function of other stem cells.[16] Bmi-1, a recently described protooncogene, is equally important to the proliferation of normal hematopoietic stem cells, and to the maintenance of proliferating leukemic cells.[17,18]

These signalling pathways (Notch, SHH, Wnt) are well-conserved mechanisms during evolution. In organisms, as *Drosophila,* and *C. elegans*, they regulate morphogenesis, while in mammals the proliferation and differentiation of various cell types at different stages of phylogenesis, ensuring the balance between the stem cells or progenitor cells and the differentiated compartment. (Obviously, these are not exclusive signalling pathways in stem cell functions but they often appear as key players.)

Wnt-pathway – This is an important factor in the regulation of cell survival. Signals are transmitted from the cell surface into the nucleus, with the help of β-catenin. If there is no signal (either the ligand or the receptor is missing) then a complex (axin, APC – a product of adenomatous polyposis coli – and glycogen synthase kinase 3b) decides the catabolism of β-catenin. In the presence of Wnt signal this complex is inactivated, β-catenin can reach the nucleus and activate certain target genes by binding to DNA. Mutations in the Wnt–catenin pathway have been observed in the development and progression of many tumor types: e.g. in epidermal stem cells of transgenic mice the continuous stimulation of the Wnt pathway can lead to malignant transformation.[19] The activation of TCF-4 (T-cell factor 4), a partner protein of β-catenin, could be considered as an early step in the development of colon cancer. In the absence of TCF-4 coding TCF712 gene the maintenance of the proliferating compartment of crypts (which „feed" the villi with cells) is disturbed. This indicates the role of TCF-4 in the regulation of stem cells located in the crypts of the normal colon.

Notch-pathway – The Notch-family consists of four cell surface receptors and at least five ligands. Binding of the ligand results in the proteolysis of the receptor, and the intracellular domain is translocated into the nucleus and will act as a transcription activator in a complex with CBF-1 (C-promoter binding factor 1). Most known target genes belong to the HES (hairy/enhancer of split) family. The HES-proteins inhibit the transcription of those basic helix-loop-helix transcription factors, that regulate cell differentiation.

The Notch-signal dictates what part of progenitor cells will be commited themselves to a certain development route and what part remains uncommitted, capable to differentiate into different cell types.[20,21] It takes part in the regulation of the set of neural stem cells.[22] Notch-receptors and ligands can be detected on the cells of different stages of development in the hematopoetic system, influencing – among others – the maintenance of stem cells and the differentiation of T lymphocytes. It is suggested that the disturbed function of the Notch-system is responsible for T-cell leukemia and breast cancers.[20]

Sonic hedgehog-pathway – The SHH-pathway is responsible for the formation of early mesoderma during ontogenesis. This molecule can activate the pathway through cell-cell contact and also as a soluble ligand. The receptor is Ptc (Patched) and probably the Smo (Smoothed) protein is also linked to it. In the absence of ligand Ptc inhibits Smo, whereas at ligand binding this inhibition ends. The activation of Smo might initiate processes during which transcription factors belonging to Gli family are activated and modify the transcription of the Ptc, WNT and Noggin – the inhibitor of BMP-4 (bone morphogenetic protein) belonging to TGF-β superfamily.[23,24] In mammals SHH regulates the proliferation of primitive hemopoetic cells through the BMP-4 molecule.[24] An error in the pathway can be a pathogenetic factor of medulloblastoma and basal cell carcinoma.[13]

The above provides examples that similar elements may take part in the regulation of stem cells and tumor cells. However, these are only indirect proofs of a close relation between stem cells and tumor cells.

Tumor cells from stem cells

If there are common ways of regulation and the errors of these pathways are important factors among the accumulated gene failure during carcinogenesis, then this gives rise to the question whether tumors originate from stem cells. One reason for this hypothesis is that stem cells have the capacity of self-renewal, and it is simpler to retain this ability than to reactivate the immortality program in an already differentiated cell. By the other argument stem cells live much longer than the differentiated ones and so they are exposed for a long period of time to impairments, collecting genetic errors leading to the breakdown of the regulation. One cannot preclude the possibility that the progenitor cells of restricted potency are the targets of carcinogenesis, but in this case they have to acquire the ability for renewal, although they may inherit it from the stem cells as a gene failure.[25]

In the hematopoetic system examples exist for both cases. The target site for tumor induction can be either the stem cell or the progenitor cell. One of the frequent chromosome alterations of AML is t:8,21 that results in the AML1-ETO protein in the leukemic cells. This gene failure was also detected in stem cells of patients in remission. Neither these stem cells nor their descendants were leukemic and in vitro they differentiated into normal myeloerythroid cells.[26] This could also mean that normal stem cells possess this mutation but more mutations, were necessary to develop leukemia. In this study the phenotype of normal hemopoetic stem cells was CD34+CD38-Thy-1+, while that of the leukemic blasts was CD34+ CD38-Thy-1-. Consequently the leukemic transformation happened either after Thy-1- progenitor cells or the stem cells lost their Thy-1 expression capacity.

The experiment when the myeloid progenitor cells' expression was influenced by hMRP-8 promoter is an example to genetic error accumulation in progenitor cells.

In transgenic mice with increased Bcl-2 expression a very similar clinical pattern formed to that of a chronic myelomonocytic leukemia but not acute leukemia. When, however, insufficiency of Fas expression is associated with this (both apoptotic pathways become impaired so the cells cannot die) 15% of the mice will develop AML.[27] (Althongh 15% is evidence without doubt, we still do not know what further changes would lead to AML in the remainder 85%. From all this one can conclude that we have more and more data at molecular level; but we know

very little about cellular responses (including interactions and heterogeneity).

Metaplastic symptoms belong to this problem. They are generally the consequence of chronic tissue damage and regeneration and indicate changes in the differentiation program. It is assumed that these changes happened in the stem cells of a particular tissue or cell type caused by different environmental factors. Causing atteration in the program, the damaging factors (like smoking induce squamous cells instead of columnar epithelium in the respiratory tract) will provoke further genetic errors in the stem cells that lead to the appearance of tumor cells.

Tumor stem cells

Tumor is regarded as abnormal tissue that grows from the descendant of a single cell through continuous accumulation of genetic errors and through different epigenetic changes. This kind of tissue consists of phenotypically heterogenic cells showing various differentiation levels. (Understanding that heterogeneity always depends on the viewpoint of the evaluation.) However, while normal stem cells have the normal organogenic program, this is defected in tumor cells. In spite of this the similarity raises the question whether all cells in the tumor is tumorigemic or not, that is: are there tumor stem cells or there are not. Here we do not mention the factors defining the progression of the tumors, which vitally influence the fate of the patient. Tumor progression is in connection with similar questions, e.g. whether all tumor cells are capable to induce metastasis or just certain tumor cells and, if the latter is true what kind of changes in geno- and/or phenotype will determine this capacity. Briefly: do the stem cells induce metastasis or not?

First it was observed in mice withemyeloma multiplex and leukemia cells *in vitro,* that only a small proportion (1:10000-1:100) of malignant cells are able of high proliferation activity that is colony formation in soft agar. *In vivo* experiments showed as well that only 1-4% of transplanted leukemia cells formed spleen colonies.[12] This phenomenon has two potential explanations: either all the tumor cells possess tumor forming capacity but at the given circumstances (in agar or in spleen) this capacity appears only in a certain part of the cells, or only a definite part of the tumor cells have unlimited proliferation potential.

In the case of human AML it was found that there is indeed some kind of hierarchy among leukemia cells. It was shown that only the CD34+CD38- population of human AML cells transplanted in immunodeficient NOD/SCID (non-obese diabetic/severe combined immunodeficiency) were capable of transmit the disease into the recipient. Conclusion: cells which can make leukemia are accumulated in 0.2% of total AML cells.[12]

Similarly to tumors of hemopoetic origin, solid tumors are also heterogenous and the cells have low clone forming capacity. There is also a vast amount of data in this respect. In this case, however, – shown by *in vivo* examinations – heterogeneity not only means difference in phenotype but also that beside tumor cells stromal cells are present very often in large numbers. In another experiment in breast trumor, a cell population was successfully separated in which cells with tumorigenic capacity accumulated. From the tumor tissue normal cells were removed with the help of markers characteristic to various developmental linage, and the remaining cells were phenotyped with CD44, CD24 (adhesion molecules), B38.1 (breast and ovarian cancer specific markers) and ESA (17-1A, epithel-specific antigen, adhesion molecule), and the cells with different phenotypes were inoculated into NOD/SCID mice.

Only ESA+CD44+CD24- cells representing 2% of the population were tumorigenic. These cells retained their tumorigenic capacity even after further passages and the phenotypic heterogeneity of the new tumor formed was alike to that of the original one. Cells differing from these phenotype could produce tumors only in a very low percentage of in the recipient. Tumorigenic cells and cells without tumorigenic capacity did not differ morphologically.[29]

The stem cells hold a great promise in tumor therapy that might be utilised in the future. Damaged cells or tissues could be replaced with them, as it has been done in case of blood-forming elements by transplanting hemopoetic stem cells. Moreover, stem cells can be used to transport various materials to tumors such as cytotoxic drug or an antitumor product modulated by gene therapy. Neural stem cells were found to posses a high migratory capacity. In animal studies these stem cells migrate through normal tissues to glioblastoma multiforme where they express the introduced genes continuously.

It is possible that the existence of tumor stem cells can be proven by experimental systems, based on tumor-forming capacity of individual cells. Although for the moment this seems to be a complicated task, the elucidation of the geno- and phenotype of normal cells gives us hope in the case of tumor cells as well. This question should be answered because it substantially influences the treatment of tumors. In case tumor stem cells exist only these has to be killed, since the other tumor cells possess limited proliferatire capacity (therefore limited life span). The only problem is that during tumor progression stem-like cells can develop continuously, and the identification, but mainly the prevention of their formation is a great challenge. The therapy should consider that stem cell function needs an appropriate microenvironment. Therefore the growth might be controlled the changing its microenvironment, as suggested by Stephen Paget's "seed and soil" theory.

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