I) Introduction

Vitally important functions that can be exemplified by the protection of the mammalian fetus from rejection, are safeguarded by multiple mechanisms. This is also true for protection against tumor development. The vulnerability of our somatic cells to potentially carcinogenic noxae and the plethora of genetic and epigenetic changes that can favor the microevolution of a cell clone towards malignancy, would make us highly cancer prone in the absence of multiple defence systems.

Traditionally, protection against cancer has been mainly if not exclusively ascribed to the immune system. The basic idea has been already expressed by Paul Ehrlich in 1909 (Ehrlich, 1909). He wrote that the complicated fetal and postfetal development must generate a large number of "aberrante Keime", translatable to "mutated cells". Were it not for the defense mechanisms of the organism, Ehrlich continued, cancer would arise in an "enormous frequency".

II) Immune surveillance

The hypothesis of immune surveillance was formulated by two prominent immunologists, Lewis Thomas in 1959 (Thomas, 1959) and Mac Farlane Burnet in 1964 (Burnet, 1971). In Burnet's words: "In large long lived animals...inheritable genetic changes must be common in somatic cells and a proportion of these changes will represent steps toward malignancy. It is an evolutionary necessity that there should be some mechanism for elimination or inactivity of such potentially dangerous mutant cells and it is postulated that this mechanism is of immunological character".

The evolutionary necessity of cancer protection is certainly true, but the unique role attributed to the immune system ignores two salient facts: a) tumor evolution involves the loss rather than the gain of many functions, and b) the cancer cell phenotype is easily malleable.

This does not augur well for the immune recognition of tumors as "non-self" targets. Even if adventitious, immunologically recognizable mutations would occur, they may be readily circumvented by further mutations or phenotypic modulation. There is one important exception, however. Oncogenic proteins of DNA tumor viruses such as SV40, polyoma, papilloma and EBV are readily recognized by the immune system as non-self. They are also relatively stable targets since their expression is a prerequisite for the proliferation of the virally transformed cell. In humans, the highly effective multicomponental protection against B cells that carry the highly transforming Epstein Barr virus (EBV) is a case in point.

If the immune system cannot mount the robust immunity envisaged in the early statements of Ehrlich, Thomas and Burnet, it is still true that we are strongly protected against cancer development. It is well established that the majority of tumor cells that disseminate during surgery do not give rise to metastasis. This is not necessarily an immune protection, however, as so frequently assumed. The well documented fact that dormant tumor cells can "wake up" years or decades later also speaks against immune killing.

What other mechanisms protect us against cancer? There is evidence for at least four different types of "non immune surveillance" against cancer. Two of them, genetic (DNA repair and checkpoint control based) and intracellular (largely apoptosis related) surveillances are well established. Evidence for epigenetic surveillance, related to chromatin structure and particularly the stringency of imprinting, has only recently started to emerge. A fourth, already quite strong and rapidly increasing
area, intercellular surveillance, points to the importance of the tumor microenvironment.

III) Genetic surveillance

Tumor risk is highly influenced by mutations in genes that control the fidelity of DNA replication, the efficacy of DNA repair, and the checkpoint controls of chromosome separation. Mutations in these genes, whether identified as point mutations, microsatellite instability, or loss of heterozygosis, are referred to as mutator mutations.

Xeroderma pigmentosum (XP) is the oldest known case of a specific DNA repair deficiency. It is due to recessive mutation in one of the essential components of the nucleotide excision repair (NER) system, the repairosome. The latter is composed of 30 different proteins, and its main function is to excise thymidine dimers from UV exposed DNA in the skin epithelium. XP patients must protect themselves from light all their lives, but they nevertheless develop multiple skin carcinomas. This points to the paramount importance of DNA repair as a first-line surveillance mechanism.

Hereditary non polyposis colon cancer (HNPPC) is due to a defect in one of several DNA mismatch repair (MMR) genes. Some of their products can splice out the mismatched region and insert new bases to fill the gap. MMR defects can be manifested as microsatellite instability (MSI) and are associated with multiple cancers. MLH1 is one of the frequently involved genes. MLH1 mutation in the hereditary cases and epigenetic silencing by dense hypermethylation of the 5' promoter region in sporadic cases can lead to the same MSI phenotype. These and other examples have identified DNA repair as a robust protection mechanism against cancer.

IV) Intracellular surveillance

Growth arrest and/or programmed cell death are best known. The former may end in apoptosis or other types of cell death. Apoptosis is the endpoint of multi-pathway, multi-step programs that lead to the enzymatic breakdown of cellular DNA. It can be initiated either through the extrinsic death receptor-ligand or the intrinsic mitochondrial pathway (for a review see Klein, 2004). Most known programs converge towards the activation of caspases that cleave cellular substrates, leading to characteristic biochemical and morphological changes. There are also caspase independent pathways of apoptosis, however. The DNA damage response, capable of acting in very early precancerous lesions, is another case in point (reviewed in Höglund, 2006).

In view of the multiple apoptotic pathways and the different levels where apoptosis can be triggered within each pathway, it may be asked whether there is a hierarchy between the different pathways. According to current consensus, inactivation events may occur in a stochastic fashion and the apparent choice between different inactivation pathways is due to selection, depending on the cell type. A certain hierarchy among the pathways cannot be excluded, however and it appears that inactivation of the Rb and p53 pathways could be a universal rule in neoplasia.

V) Is there epigenetic surveillance?

Several findings speak for an affirmative answer. The normally inactivated maternal allele of the IgF2 gene showed loss of imprinting (LOI) in about 10% of the normal human population (Cui et al., 2003). This LOI was associated with a 3.5-5 fold increase in the risk of colorectal adenoma development. This surprising finding has been corroborated in a mouse model system (Sakatani et al., 2005). Hybrid mice were generated by crossing two genetically engineered mouse strains. The females used for the cross were heterozygous for a deleted differentially methylated region (DMR). Inheritance of this deletion from the mother leads to the biallelic expression of IGF2 - corresponding to loss of imprinting. The males entering the hybrid cross were of the Min strain that carries a mutation in the adenomatous polyposis coli (APC) gene. APC mutations provide a strong predisposition to familial colon polyposis, a precancerous condition in humans and mice.

All hybrids derived from this cross carried the APC mutation, but only half of them inherited the imprinting defect. The frequency of intestinal adenomas was twice as high in the mice with the imprinting defect than in their littermate controls. Also, their intestinal crypts were longer and showed increased staining for proteins characteristic of intestinal-cell progenitors. Differentiation of the crypt cells to more specialized intestinal cell types was delayed.

These observations show that the impairment of normal prenatal imprinting may interfere with cellular differentiation and thereby increase the probability of cancerous development.

In more general terms, they suggest that cancer susceptibility may be influenced by differences in the stringency of epigenetic control.

This is consistent with earlier work showing that inbred mouse strains differ in the activity of enzymes involved in DNA methylation (Paz et al., 2002).

VI) Intercellular surveillance

This can also be referred to as microenvironmental control. A wide variety of observations fall into this category. The earliest experimental reports are based on the contactual interaction between tumor and normal cells. Authors like Michael Stoker, Leo Sachs, Harry Rubin and others have shown already in the 1960s, that admixture of normal to tumor cell suspensions can dramatically decrease the focus or
colony forming efficiency of the tumor cells. Cell contact is required for the effect. Recent studies have identified some of the junctions involved (for review see Glick and Yuspa, 2005). Adherence junctions play an important role. E-cadherin, a major structural component of the adherence junctions, is downregulated in most epithelial tumors, usually by promoter DNA methylation. Structural constituents of the adherence junctions, like catenins or connexins, are frequently mutated. Reestablishment of cadherin expression by transfection can revert the tumor phenotype.

Other structural components of the tumor cell membrane may be involved in contactual control as well. β-integrins are often abnormally expressed by tumor cells. Antibody targeting of a rearranged, tumor associated β-integrin could inhibit tumor growth (Weaver et al., 1997). Notch receptors and their ligands regulate differentiation and proliferation. Their deletion in the basal layer of mouse epidermis leads to epidermal hyperplasia and skin tumor. Notch signaling between normal and pre-neoplastic cells can contribute to the suppression of the neoplastic phenotype (Glick and Yuspa, 2005).

These and other contactual controls between normal and tumor cells may also explain the previously mentioned observation, that many disseminated tumor cells never grow into metastatic tumors. In one experimental model (Naumov et al., 2002), it was found that a significant fraction of injected mouse mammary tumor cells of either high or low metastatic potential persisted as solitary non-dividing cells in the liver. Reinoculated to new hosts they were fully tumorigenic. Similar dormancy of solitary tumor cells has been observed with melanoma, squamous cell carcinoma and prostate carcinoma cells. The “awakening” of the dormant tumor cells may be accelerated by disturbing the tissue equilibrium e.g. by phorbol esters.

The effects of the microenvironment on tumorigenicity are not restricted to contactual controls. Certain tumor cells can be induced to differentiate and loose tumorigenicity following their exposure to natural or non-natural signals. The most spectacular experiment in this category is the demonstration by Beatrice Mintz that cells of a highly malignant, but diploid teratoma could be “normalized” by implantation into early mouse embryos (Mintz and Illmensee, 1975). Microenvironmental structure may also exert a profound influence. Tumorigenic 2D cultures of mammary carcinoma cells could loose their tumorigenicity partly or completely, after they have been built into a 3D acinar structure in vitro (Nelson and Bissell, 2005).

The tumor environment can thus influence the propensity of neoplastic cells to proliferate in vivo in a number of different ways. Some act by direct contact between tumor and normal cells, while others may act in a more distal, signal mediated fashion.

VII) Own experiments

As an experimental approach towards the study of the inhibition of tumor progression by the normal stroma, we have chosen to study the phenomenon of neighbor suppression, described by Stoker et al. (Stoker et al., 1966). Our results so far obtained have been published in two papers (Flaberg et al., 2011; Flaberg et al., 2012).

In the first study, the effect of 107 samples of low passage number primary normal fibroblasts from pediatric and adult donors was tested on the growth of six human tumor cell lines. The majority of the tested fibroblasts inhibited the proliferation of the tumor cells. The proliferation inhibiting effect of the fibroblasts differed, depending on the site of origin. Skin fibroblasts were more inhibitory than prostate fibroblasts, derived from donors with prostatic cancer. Normal hernia fibroblasts were less inhibitory than skin fibroblasts.

Inhibition required direct cell contact. The inhibitory effect could also prevail across the mouse - human species barrier.

The second study showed that effective inhibition requires the formation of a morphologically intact fibroblast monolayer before the seeding of the tumor cells. Interference with the formation of the monolayer impaired the inhibition.

Telomerase immortalized human fibroblasts were good inhibitors. Based on morphological criteria, more and less inhibitory subclones could be selected from the telomerase immortalized line.

Comparison of highly inhibitory and less inhibitory fibroblasts from the in vitro immortalized line and of inhibitory and less inhibitory ex vivo explants, identified a set of genes that co-segregated with the inhibitory phenotype.

This was taken to suggest that our model system may reveal molecular mechanisms involved in contact mediated microenvironmental surveillance.

References


Burnet FM., Immunological surveillance in neoplasia. Transplant Rev. 1971;7:3-25.


Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ., Reversion of the malignant


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