Toward a genetics of cancer resistance

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Two of three humans never get cancer. Even the majority of heavy smokers remain cancer free. Is this a matter of chance, or are there cancer-resistant genotypes? Based on the evidence discussed, it would appear that evolution has favored a limited number of relatively common resistance genes that may nip incipient cancerous foci in the bud, i.e., to stop them at their inception. It is further suggested that resistance genes may act at the level of tissue organization in a dominant fashion.

The devastating effects of cancer are highly visible in the human population. Approximately one in three persons is struck by neoplastic disease at one time or another. It is less often emphasized that two of three persons remain unaffected. Even the majority of heavy smokers, who bombard their lungs with carcinogens and tumor promotors over many years, remain cancer free. In view of the many genetic and epigenetic changes that can promote cancer development and/or progression (1), this is quite surprising.

Naturally, the suffering of the cancer patients and their families has stimulated most cancer researchers to focus on the cellular changes in cancer and on the genetics of cancer susceptibility. The genetics of cancer resistance, as a topic in its own right, has remained largely unexplored. But, one may ask, is cancer resistance not just the other side of the susceptibility coin? If cancer development is favored by the mutation or the allelic polymorphism of many genes involved in the control of cell differentiation or division, is cancer resistance not simply the result of an accidental low occurence of such mutations or the low prevalence of cancer-favoring alleles? Maybe so, but there is another alternative. Here it shall be argued that the genetics of cancer resistance needs to be studied on its own merits and not merely as a mirror image of cancer susceptibility.

Results and Discussion

Evolutionary Considerations. The predominant occurrence of cancer in the older age groups is often attributed to the inability of natural selection to favor protective mechanisms against diseases that occur after the end of the reproductive age. This may sound plausible, but it is confounded by the fact that the reproductive age has no sharp upper limit in males. In wild populations, a small number of dominant males tend to father most of the offspring until challenged by younger competitors. This has been well documented in confined mouse populations (e.g., in silos) and in free-living primate colonies with monarchic or oligarchic social structures. The predominant occurrence of cancer in the older age groups may have explanations other than the relaxation of natural selection. It may reflect the multistep nature of cancer development. Five to seven successive stochastic changes in the same subclone may take considerable time.

As argued in the following sections, there are good reasons to believe that the relative cancer resistance of our species is secured by robust protective mechanisms that counteract the development and/or progression of neoplastic cells.

Cancer Resistance in Mice. The establishment of the well-known high cancer strains by selective breeding during the first part of the last century and the genetic analysis of their susceptibility has overshadowed the important fact that low cancer strains could be established with equal ease by the opposite selection.

It is also noteworthy that murine subspecies of feral origin, such as *Mus spretus* and *Mus castaneus* have a low tumor incidence (2). F1 hybrids of *M. spretus* and *Mus musculus* were highly resistant to most chemical carcinogenesis protocols. In contrast to some strains of *M. musculus* controls, the hybrids failed to develop tumors in the skin, liver, colon, lung, or the lymphoid system (3). The prediction that *M. spretus* carries multiple tumor resistance genes has been confirmed by the mapping of several loci that convey resistance at different stages of skin (4) and lung (5) tumor development.

Are There Human Low Cancer Families? Epidemiological evidence indicates that a significant proportion of the human population is highly resistant and that an equally significant fraction is highly susceptible to cancer (6). With the unique Icelandic genealogic database containing information on Icelandic families dating back several hundred years, Iceland has excellent conditions for focused studies on cancer resistance. One such study is presently planned by the Icelandic Cancer Registry. Through the cohort study of the Cancer Detection Clinic, the aim is to identify 200 cancer-free women who have reached old age and are long-term smokers. The family trees of these 200 women will then be traced and the risk of cancer in these families compared with that in the general population; members of families with an unusually low risk of cancer (SIR <0.2) will then be selected for further study on intercellular cancer surveillance [see *Intercellular Surveillance (Microenvironmental Control)*].

Mechanisms of Resistance. In an earlier text (7), we have listed five mechanisms with proven anticancer surveillance function: (*i*) immunological, acting mainly against virus-induced or virus-associated cancer; (*ii*) genetic, a large set of robust DNA repair mechanisms; (*iii*) epigenetic, based on the stringency of imprinting; (*iv*) intracellular, reflected by the triggering of apoptosis in cells with illegitimately activated oncogenes or with extensive DNA damage; and (*v*) intercellular, also referred to as microenvironmental control (discussed in more detail below). A possible sixth mechanism is suggested by the discovery of HAMLET, a protein-lipid complex formed by the interaction between a component of human milk and a lipid cofactor at the acid pH in the stomach of the breast-fed child. HAMLET induces p53-independent apoptosis in tumor cells but leaves differentiated cells unaffected (8).

Among the six mechanisms, the genetic and the intracellular mechanisms of resistance, acting through DNA repair and through the triggering of apoptosis, respectively, are presently known as the most powerful protection mechanisms. This is also reflected by the significantly increased tumor frequencies in correspondingly deficient individuals.

Genetic Variation in Cancer Surveillance. The immunological, genetic, epigenetic, and apoptosis-triggering surveillance mechanisms are known to be influenced by genetic variation, as exemplified

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below, whereas the microenvironmental control of tumor growth has not been studied from the genetic point of view.

"Immunological Anticipation." Efficient immune rejection of tumors requires T-cell clones with specific receptors that can target non-self proteins. Unequivocal evidence for such targets is available only for virus-induced or virus-associated tumors. In humans, they can be exemplified by Epstein-Barr virus (EBV)-driven immunoblastomas (9, 10), human papillomavirus-carrying epithelial or cervical tumors (11), and human herpesvirus-8-carrying Kaposi sarcomas (12). EBV-driven immunoblastomas have been most extensively studied. They arise in patients whose immune systems have been impaired congenitally, iatrogenically (e.g., in transplant recipients), or by HIV infection (13).

Among the genetic deficiencies, the X-linked lymphoproliferative syndrome (XLP) is particularly interesting, because it is usually caused by a mutation in a small immunoregulatory protein (SAP) (14). For reasons that are not well understood, this leads to a relatively specific impairment in the efficiency of the immune system to keep EBV-carrying B-lymphocytes under control.

The oncogenic herpesviruses can also illustrate another immune surveillance-related point. Potentially oncogenic viruses that are ubiquitous in their natural host, such as EBV in humans, *Herpesvirus saimiri* in squirrel monkeys, and *Herpesvirus ateles* in spider monkeys, have selected their hosts for a high level of resistance, not against the virus infection as such but against its tumorigenic consequences. Tumor development occurs as an accident of immunodeficiency, as already discussed for EBV. The high resistance of the immunocompetent human host against EBV is reflected by the readiness of the T-cell system to respond to virally infected cells (15). Confrontation of EBV-infected B cells with autologus T-cells generates T-cell-mediated cytotoxicity and lymphokine release equally promptly and at a comparably high level as confrontation between MHC incompatible cells. I refer to this readiness as "immunological anticipation." It is also reflected by the unusually high proportion of T cells that can bind tetrameric complexes of virally encoded antigens and appropriate MHC molecules (16). Conceivably, EBV may have started out as a highly pathogenic virus, following the separation of the Old and New World primates about 40 million years ago, as it still is in the immunologically naive New World primates. Subsequently, selection of the host and the virus has led to the present nearly nonpathogenic equilibrium in the Old World primates.

Immunological anticipation can be seen in the historical context of this and other virus-host relationships. The oncogenic potential of EBV, *H. saimiri* and *H. ateles*, neither of which cause tumors in their immunocompetent natural host, is readily manifested in primates that do not normally encounter these viruses. All three can induce fatal lymphoproliferative disease, e.g., in the marmoset (17). In an early comparative study of the antibody response to *H. saimiri* in the tumor-resistant squirrel monkey and the susceptible marmoset, we have found a striking difference in the timing of the response (18). Antibodies rose to a high level 3 days after the infection in the previously uninfected squirrel monkey but only after 2–3 weeks in the marmoset. By that time, however, the marmosets already had rapidly expanding lymphoproliferative disease.

The potential for immunoselection is also illustrated in a related oncogenic herpesvirus system. The lymphotropic Marek disease herpesvirus (MDHV) is not ubiquitous in its natural host, the chicken. Before the present efficient vaccination policy, it was manifest by enzootic outbreaks of lymphoproliferative disease. Resistant flocks could be readily selected, however. Resistance was often linked to major histocompatibility complex class I, indicating the importance of T-cell-mediated responses (19).

The immune system finds no clearly defined non-self targets in the nonviral tumors. With the possible exception of malignant melanomas, it regards the cells of most spontaneous tumors as "self." Some cancers express "cancer-testis antigens" that are also present in the testis but not in normal adult somatic tissues (20). Targeting them and other tissue antigens by immune effectors is being intensely explored in many laboratories. Most approaches amount to the breaking of tolerance and must run against the "horror autotoxicus," another term coined by Ehrlich, referring to the robust defense system against autoimmune reactions.

Genetic Variation in DNA Repair. Tumor risk can be influenced by mutations in genes that control the fidelity of DNA replication, the efficacy of DNA repair, and the checkpoint controls involved in DNA synthesis and chromosomal mechanics. Mutations in such genes are often referred to as *mutator mutations.*

Xeroderma pigmentosum is the oldest known case of a specific DNA repair deficiency. It is caused by recessive mutation in one of the essential components of the nucleotide excision repair (NER) system, the repairosome, composed of 30 different proteins (21). Its main function is to excise thymidine dimers from ultraviolet light-exposed DNA in the skin epithelium. Persons with xeroderma pigmentosum must protect themselves from light all their lives, but they nevertheless develop multiple skin carcinomas. This highlights the paramount importance of DNA repair as a front-line surveillance mechanism.

Hereditary nonpolyposis colon cancer is caused by a defect in one of several DNA mismatch repair genes. Normally, their protein products can splice out the mismatched region and insert new bases to fill the gap (22). Mismatch repair gene defects can manifest themselves as microsatellite instability and are associated with multicancer syndromes. *MLH1* is one of the frequently involved genes (23). Its mutations in the hereditary cases and its epigenetic silencing by dense hypermethylation of the 5′ promoter region in sporadic cases can lead to the same microsatellite instability phenotype (24).

In addition to the direct evidence from these and other deficiencies, showing that DNA repair is a robust protection mechanism against cancer, it is also clear that there are individual variations in the efficiency of its many components. Only a few examples will be mentioned. Oxidative repair genes that can prevent mutagenesis and tumor formation are a case in point. The human DNA repair genes mut1 (Myh in mice), and mutM (Ogg1 in mice) prevent G-to-T mutations, caused by reactive oxygen species and generated as byproducts of normal metabolism. Myh and Ogg1 deficiencies have led to a variety of tumors in the majority of knock-out mice (25). In combination with the heterozygosity of Msh2, a mismatch repair gene, these deficiencies had a synergistic effect in generating malignant lung tumors.

Genetic polymorphisms in the same and other DNA repair genes may influence cancer risk in humans as well. For example, the Cys-Cys isoform of the Ogg1 gene is associated with decreased base excision repair and increased lung cancer risk (26). XRRC1, which belongs to the x-ray repair cross-complementing group 1, is also polymorphic. The 194 Trp form is characterized by a relatively low mutagen sensitivity and provides a certain degree of protection against tobacco-related cancers. In contrast, the 399Gln form is associated with higher mutagen sensitivity and increased risk of tobacco-related cancers in light smokers.

Apoptotic Propensity. Both DNA damage and illegitimate activation of oncogenes such as myc, ras, E1A, and E2F can trigger apoptosis through a variety of pathways. Apoptotic propensity can be influenced by genetic variation. A single-nucleotide polymorphism in the MDM2 gene, an antagonist of p53 and thereby an important regulator of the p53-dependent apoptotic pathway, is a case in point (27). Compared with the wild type (TT), the GG allele of SNP 309 creates a strong binding site for

the transcription activator SP1, leading to increased expression of MDM2. This attenuates the p53 pathway, leading to higher incidence and earlier onset of tumors in Li-Fraumeni patients who carry p53 germline mutations. These individuals develop soft-tissue sarcomas, breast cancer, and other tumor types 10–12 years earlier than corresponding patients with a TT genotype. Other polymorphisms within the p53 pathway that reduce its apoptotic efficiency affect the AKT upstream regulator and the protein itself (28).

Is There Genetic Variation in Oncogene Activation? There is not much information on this point. A possible example is mouse plasmacytoma (MPC) induction by the intraperitoneal injection of mineral oil. The BALB/c strain is eminently susceptible to this mode of tumor induction, whereas most other strains are resistant (29). BALB/c appears to be particularly prone to the activation of the myc oncogene by juxtaposition to one of the three Ig loci, brought about by chromosomal translocation, a *conditio sine qua non* for MPC induction. Using Emu-IL-6 transgenic mice, Kishimoto *et al.* have shown that the probability of the Ig/myc translocation may be genetically variable (30). Interleukin-6 is a powerful stimulator of plasma cell proliferation. Mice of the MPC-resistant C57BL strain that carried an Emu-IL-6 transgene developed benign plasmacytosis but no plasmacytomas. On a BALB/b background, the same transgene induced genuine plasmacytomas that carried the translocations. This suggests that the Ig/myc translocation is more likely to occur in BALB/c than in C57BL mice. However, there is, in addition, a difference in suppressor gene inactivation. The p16 isoform carried by BALB/c is functionally weaker then the wild type, and it is less able to trigger growth arrest within the Rb pathway (31). Weakening of this powerful tumor suppressor mechanism may contribute to the high susceptibility of BALB/c to MPC-genesis.

For the human counterpart of the Ig/myc translocation carrying MPC, Burkitt lymphoma (BL), there is no evidence for any genetic variation in the probability of the translocation itself, but the question has not been properly studied. The rescue of the translocation carrying, apoptosis-prone cells may be similar in the two systems, however. The Ig/myc translocations are known to arise spontaneously, as accidents of the physiological Ig-gene rearrangement. Cells with constitutively activated myc are driven to apoptosis unless rescued by appropriate survival factors. They may be provided by B-cell stimulatory cytokines. The mineral oil induced granuloma that is a necessary prerequisite for MPC development (29) is a rich source of such cytokines. Ig/myc translocation carrying BL and BL-like lymphomas are most frequently observed in conjunction with chronic hyperendemic malaria or human immunodeficiency virus infection. Both conditions are associated with high levels of B-cell stimulatory cytokines. After rescue of the translocation-carrying clone, the lymphokines can also stimulate it to expand. This opens the way for the secondary changes that regularly occur in BL, namely, crippling of the Rb pathway, usually by p16 methylation and of the p53 pathway by p53 mutation, ARF deletion, or MDM2 amplification (32).

Variation in Epigenetic Imprinting. Cui *et al.* have shown that the maternally imprinted IGF2 gene loses its imprinting in about 10% of the normal human population (33). The resulting biallelic expression of the gene was associated with a 3.5- to 5-fold increase in the frequency of colorectal tumors. This unexpected finding was corroborated in a mouse system (34). Hybrid mice were generated by crossing two genetically engineered strains. 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 for colonic polyposis, a precancerous condition in humans and mice. The females used for the cross were heterozygous for a deleted differentially methylated region linked to the IGF2 gene. Inheritance of this deletion from the mother leads to the biallelic expression of IGF2 in the hybrid offspring, corresponding to the loss of imprinting in the human population. All hybrids derived from the cross thus 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 as compared with their littermate controls. Moreover, their intestinal crypts were longer and showed increased staining for early intestinal-cell progenitor markers, indicating that the differentiation of the crypt cells into more specialized intestinal cell types was delayed.

The inference that impairment of normal parental imprinting may interfere with cellular differentiation and may thereby increase the probability of cancerous development was also corroborated by the finding of Holm *et al.* that the global loss of imprinting could lead to widespread tumorigenesis in adult mice (35). They generated imprint-free mouse embryonic stem cells (IF-ES cells) by transient demethylation. Embryonic fibroblasts derived from the IF-ES cells showed reduced p19 and p53 expression. These cells grew as immortal cells *in vitro* and were tumorigenic in SCID mice. Chimeric animals derived from the IF-ES cells developed multiple tumors, which all arose from the injected IF-ES cells. Moreover, the imprinting defect acted synergistically with H-ras in cellular transformation.

Intercellular Surveillance (Microenvironmental Control). The existence of this mechanism or, more appropriately, this conglomerate of mechanisms, has been well documented, but its genetics have not been investigated. An important early finding was made by Stoker *et al.* in 1966 (36). These investigators found that normal cells could inhibit the growth of neighboring polyoma-transformed cells by direct contact. Similar findings were made with oncogene (myc, ras, src)-transformed cells (37). Furthermore, Borek and Sachs (38) reported a certain "hierarchy" in the ability of different transformed cells to inhibit each other and to be inhibited by normal cells.

In more recent years, the field has been extended to organ cultures of epithelial and connective tissue. It was confirmed that the survival and growth of labeled tumor cells was inhibited by contact with normal epithelium or stroma (39, 40). Direct cellular contact is mandatory, and adherence junctions appear to play an important role. This is consistent with the fact that E-cadherin, a major structural component of the adherence junctions, is downregulated in most epithelial tumors, usually by promoter DNA methylation. Other components of the adherence junctions, such as catenins or connexins, are frequently mutated. Re-establishment of cadherin expression by transfection can revert the tumor phenotype.

Other structural components of the cell membrane may be involved in microenvironmental inhibition of tumor growth as well. β-Integrins are often abnormally expressed by tumor cells. Antibody targeting of a tumor-associated, structurally changed β-integrin was found to inhibit tumor growth (41). Notch receptors and their ligands that regulate differentiation and proliferation is another example. Their deletion in the basal layer of mouse epidermis can lead to epidermal hyperplasia and skin tumors (39).

Such, and other, contactual interactions between normal and tumor cells may be at least partly responsible for the frequent observation that the majority of most disseminated tumor cells never grow into metastases. In one experimental model, a significant fraction of injected murine mammary tumor cells of either high or low metastatic potential persisted as solitary nondividing cells in the liver (42). These cells were fully tumorigenic when reinoculated into new hosts. Dormancy of solitary tumor cells has also been demonstrated with melanoma, squamous cell carcinoma, and prostatic carcinoma cells in the same

model. "Awakening" of the dormant tumor cells could be accelerated by altering the tissue equilibrium, e.g., by exposure to phorbol esters.

The effects of the microenvironment on tumorigenicity surfaced in many other experimental systems. Certain tumor cells could be induced to differentiate and to lose tumorigenicity by exposing them to natural or non-natural signals. As an extreme case, Mintz and Ilmensee showed that cells of a highly malignant but diploid teratoma could be "normalized" by implantation into early mouse embryos (43). This spectacular experiment has been neither confirmed nor contradicted in the molecular era.

In less dramatic but possibly analogous systems, the reconstruction of near-normal tissue architecture inhibited the growth of anaplastic tumor cells. Tumorigenic two-dimensional cultures of mammary carcinoma cells could lose their tumorigenicity, at least temporarily, after they had been built into a three-dimensional acinar structure *in vitro* (44). Similar experiments have been performed on liver/hepatocarcinoma systems. Normal cells of the precancerous liver were less able to inhibit the outgrowth of hepatocarcinoma cells than cells of normal liver. A similar difference was found in epithelial cultures in which normal epithelial cells, but not papilloma cells, could inhibit the growth of neighboring cancer cells.

A particularly striking case of microenvironmental control was shown to curb the transforming effect of a powerful oncogene. In the pivotal experiments of Peyton Rous in 1911 (quoted in ref. 1), the "filterable" agent that was to be called Rous sarcoma virus (RSV) induced tumors at the site of inoculation in newly hatched chicks after a relatively short latency. This transforming activity could later be attributed to the v-src oncogene. However, when chicken embryos were infected with v-src at an early embryonic stage, they developed normally. After hatching, the birds developed no tumors. On culturing, their fibroblast grew immediately with a transformed phenotype. Moreover, wounding the v-src-carrying birds made tumors appear at the site of tissue regeneration (45). It was concluded that the puncture wound must have been essential for the induction of tumors in Rous's original experiment. Apparently, the healing process had led to the breakdown of microenvironmental controls that normally inhibited the growth of the transformed cells.

Working with the same v-src-transformation system, Alexander *et al.* (46) showed that the suppression of transformed cell growth by neighboring nontransformed cells required direct physical contact; communication by diffusible factors was not sufficient. Importantly, the src-kinase activity of the transformed cells was not inhibited by the contact with the nontransformed cells. The inhibited tumor cells showed increased expression of the LIMD1 tumor suppressor gene, and of serum deprivation response protein. The growth-suppressed tumor cells retained their ability to grow as transformed cells upon reisolation from the inhibited cultures. Gap junctional communication was not essential for the contractual inhibition of transformed by normal cells, because the effect persisted when the normal cells were taken from gap junction knockout mice. The postulate that adherence junctions were important was consistent with the finding that reexpression of E-cadherin could suppress the transformed phenotype in isolated cancer cells (47), whereas the blocking of cadherin function by a dominant negative mutant enhanced invasion (48).

The histopathologically observed lack of progression of numerous microscopically detectable cancerous foci in, for example, the prostate or the mammary gland in persons who never develop clinical tumors may be caused by similar controls. The persistence of epithelial cells with defined lesions in their oncogene and/or tumor suppressor gene equipment that failed to grow while in contact with normal epithelium is another case in point (49). Suppression of initiated preneoplastic or neoplastic cells by normal cells can be overcome by a variety of noncarcinogenic agents, classed as tumor promotors (50).

The exploration of genetic and/or developmental (e.g., age-dependent) variations in the efficiency of the contactual inhibition of neoplastic by normal cells is urgently needed. I would not be surprised if it would turn out that this inhibition is a major factor in tumor resistance and its breakdown in tumor susceptibility. Indirect but suggestive evidence comes from a group of studies in the 1950s that are now largely forgotten. Six different groups of cancer researchers, using different tumor systems, applied the principle, newly discovered at the time, that F1 hybrids between two homozygous mouse strains can serve as a universal recipients of tissue transplants from both parental strains, as they carry the histocompatibility gene equipment of both parents. As summarized by Heston (51), crosses were made between a highly susceptible and a resistant strain with regard to mammary carcinoma, lung adenoma, testicular tumor, adrenal tumor, and thymus-derived lymphoma. (The experiments on the lung tumor system were carried out on dimethylbenzanthracene-treated mice and the experiments on the thymus on x-ray-treated mice). In each case, the normal tissue, lung, mammary gland, testis, adrenals or thymus, respectively, were transplanted subcutaneously from the high and the low cancer strain parent to the two flanks of the common F1 hybrid recipient, whose corresponding normal tissue (mammary [glands], testis, adrenals, thymus) had been removed. The graft healed and the tumor frequencies could now be compared in the same host. The incidence of tumors in the tissue derived from the high-tumor parent was much higher (10-fold or more) compared with the tissue from the low-tumor parent. This showed that selection for high or low cancer incidence in a given tissue has not only fixed genes that influence cancer incidence at the level of the host (hormonal, viral, immunological) but also genes that influence the propensity of cancer development in the target tissue itself.

Informative as they are, the F1 experiments do not tell us whether those genetic differences act at the level of the parenchyma, the stroma, or the microenvironmental interaction between the two. A recent experiment, conducted in a different system, showed that the stroma can be of paramount importance. Maffini *et al.* (52) used a chemically induced rat mammary tumor system and the directly acting carcinogen *N*-nitrosomethylurea (NMU). The mammary fat pad of female recipients were cleared of glandular tissue, which was explanted *in vitro* and then reimplanted into the cleared fat pad, with or without exposure to NMU. Exposure of the cleared fat pad to NMU before the implantation of the gland led to tumor development in the untreated cells of the mammary epithelium, whereas the implantation of NMU treated glands to untreated fat pads did not. This would suggest that the carcinogen exposure has affected a microenvironmental control system that would normally prevent the development or progression of precancerous or cancerous cells.

Suppression of Tumorigenicity by Somatic Hybridization. Together with Henry Harris, our group has tested a large series of somatic hybrids generated by the fusion of normal with tumor cells for tumorigenicity *in vivo* (reviewed in ref. 53). The combinations involved carcinomas, sarcomas, and lymphomas as the malignant parents, and fibroblasts, epithelial cells, or lymphocytes as the normal parents. The tumorigenicity of the hybrids was suppressed, as a rule, as long as they maintained most chromosomes from the normal parent. The *in vitro* growth of the hybrid cells was not suppressed, however. This was no surprise, because the experimental design was based on the selection of continuously growing somatic hybrid lines *in vitro* that were tested for tumorigenicity. This selects automatically against the expression of tumor suppressor genes derived from the normal parent cell that would induce apoptosis or growth arrest *in vitro*.

The question how the normal-parent-derived tumor suppres-

sors act has not been clarified. Receptors for contactual microenvironmental control are among the obvious candidates. Exploration of this uncharted system may lead to a hitherto unknown world of tumor suppressors. The molecular and tumor growth-related functional analysis of chromosme regions that are frequently or regularly lost from the short arm of human chromosome 3 in the course of tumor growth has already identified several "asymmetric tumor suppressor genes" that can inhibit the *in vivo* but not the *in vitro* growth of both murine and human tumor cells (7). Examples include the lactoferrin (LF), LIMD1, HYAL1, and HYAL2 genes (54).

Conclusion

The genetics of tumor resistance, responsible for the protection of the majority of individuals against cancer development, is the great *terra incognita* of cancer genetics. Among the known protective mechanisms against cancer, intercellular surveillance or microenvironmental control is crucial for the understanding of tumor cell dormancy, prolonged latency of initiated cells, and the nipping of microtumors in the bud (i.e., stopping them at their inception), in organs such as the prostate or the mammary gland. Conceivably, evolution may have favored a limited number of relatively common resistance alleles that may act at the level of tissue organization in a dominant fashion. It will now be very important to test whether the ability of normal cells to inhibit tumor cells by direct contact is subject to genetic and/or developmental variation. In addition, identification of genes that inhibit *in vivo* tumorigenicity but not *in vitro* growth, pioneered by early studies on somatic hybrids between normal and tumor cells, may open the gates to a hitherto unexplored world of tumor suppressor genes.

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