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Review

Genetic predisposition to sporadic cancer: How to handle major effects of minor genes?

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Abstract. Predisposition to non-familial, sporadic cancer is strongly influenced by multiple tumor susceptibility genes (TSGs), each with apparently minor effects on the cancer phenotype. Sequence analysis of the human genome has yielded numerous single nucleotide polymorphisms (SNPs), raising the expectation that new low-penetrance TSGs will be identified that can be used to estimate an individuals cancer risk. However, mouse models for human cancer showed that the effects of many low-penetrance TSGs are highly variable due to their involvement in epistatic interactions. Together, these interacting TSGs form large molecular networks, which represent cancer-associated biological modules that influence the tumorigenic process. As a consequence, although allelic variation in one TSG on a permissive genetic background can have major effects on tumor development, the net effect of allelic variation in multiple interacting TSGs remains hard to predict. Therefore, the predictive value of SNP-analysis to estimate an individuals cancer risk will be restricted to those TSGs that exhibit single-gene effects. New strategies need to be developed to evaluate cancer risk associated with biological modules that are influenced by TSG-networks.

Keywords: Genetic predisposition, tumor susceptibility genes, epistasis, sporadic cancer, mouse

List of frequently used abbreviations: TSGs, tumor susceptibility genes; SNPs, single nucleotide polymorphisms; FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; RC, recombinant congenic; PLA_2, phospholipase A_2; IL, interleukin.

1. Introduction

In past decades revolutionary developments in the field of molecular biology have greatly improved our understanding of the fundamental processes underlying tumor development. Although this knowledge has revealed new targets for anti-tumor therapy, cancer is still a leading cause of mortality in the Western world. Early diagnosis of cancer remains one of the best predictors to cure from this disease, in particular when pre-malignant lesions are discovered and removed before they acquire malignant, metastatic properties. Several countries run organized population-wide screening programs for breast cancer, cervical cancer, and more recently also colorectal cancer, aiming to reduce mortality rates by systematic screening of all individuals at average risk [17,50]. Cost-effectiveness of these screening programs is under continuous debate [41]. The efficiency of organized screening programs would improve significantly when an individuals genetic predisposition to sporadic cancer could be taken into account [58]. However, genes that modify susceptibility to sporadic cancer appear to have only minor effects, and their identification from the human population is extremely difficult. Surprisingly, studies that used mouse models of human cancer not only confirmed the existence of numerous TSGs, but also demonstrated that their individual effects are highly underestimated. This review aims to summarize why 'minor' TSGs can have 'major' effects on genetic predisposition to sporadic cancer, and to indicate how this knowledge can be used for development of new strategies to improve estimation of an individuals cancer risk.

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2. Tumor susceptibility genes

It is estimated that the frequency of SNPs in the human genome is about one per 1000 base pairs, resulting in about ~3,000,000 SNPs that determine genetic variability of the human population. Some of these polymorphisms are responsible for allelic variation in TSGs and lead to phenotypic differences in susceptibility to cancer between individuals. 'Classical' genetic approaches are a powerful tool to identify those polymorphisms in the genome that are *causally* responsible for variation in disease phenotype, without prior knowledge or prejudice about the function of susceptibility genes that affect disease etiology.

2.1. Highly penetrant hereditary cancer syndromes

Familial cancer is defined as cancer that occurs within families at relatively high frequency and at low diagnostic age compared to the general population. Familial clustering of cancer is frequently caused by hereditary factors with strong effects. For example, patients that suffer from familial adenomatous polyposis (FAP) develop hundreds of polyps in their intestines because they carry one cancer-associated allele of the adenomatous polyposis coli (*APC*) tumor suppressor gene in their germline. The penetrance of such *APC* alleles towards colon cancer is very high, approaching 100%. Therefore, susceptibility to colon cancer within FAP families is inherited in a Mendelian fashion as a monogenic trait, and polymorphisms in the *APC* gene that cause FAP could be identified using a straightforward genetics approach. First, the *APC* locus was mapped to human chromosome 5q21 by linkage analysis, upon which positional cloning procedures revealed that germline alterations in the *APC* gene were responsible for the FAP phenotype [42,56]. Once identified as causative for hereditary polyposis associated colon cancer it soon became apparent that the *APC* tumor suppressor gene also plays a common and critical role in the etiology of non-familial, sporadic colon cancer.

The example of identification of the *APC* gene as the cause of FAP illustrates that genetic approaches result in identification of genes that cause variation in disease susceptibility. Once a gene is identified as a susceptibility gene for cancer, further analysis of its function may open a whole new area of research that provides novel insights into the molecular mechanisms that are underlying tumorigenesis. The most common hereditary cancer syndromes and the high-penetrance susceptibility genes involved were recently reviewed by Nagy et al. [55].

2.2. Sporadic cancer

More than 90% of cancer cases consist of non-familial, sporadic forms of cancer [62]. Compared to the risk for cancer in the general population, the relative risk of family members of patients with sporadic tumors only appears to be 2- to 4-fold increased [24], which does not encourage the idea that predisposition to sporadic cancer is influenced by hereditary factors with strong effects. However, the observed relative risk is much smaller than the real difference in relative risk between genetically susceptible and non-susceptible individuals, which is estimated to be 10- to 100-fold. The observed relative risk is obscured by the facts that: (1) Not all cancer patients are genetically 'susceptible' individuals; (2) Even fewer of their relatives are genetically 'susceptible' individuals; and (3) The general population is a mix of genetically 'susceptible' and 'non-susceptible' individuals [57]. Indeed, a more recent study of non-familial breast cancer estimated that the 20% individuals at highest risk may be up to 40-fold more susceptible to cancer than the 20% individuals at lowest risk [58], indicating that predisposition to *sporadic* cancer is strongly influenced by genetic factors. However, in contrast to hereditary cancer syndromes that are dominated by the effect of a single gene, genetic predisposition to sporadic cancer is multifactorial. Identification of low-penetrance TSGs by linkage analysis and positional cloning procedures is hampered by genetic and environmental heterogeneity of the human population. Instead, association studies have been performed with polymorphisms in genes that are likely to affect the tumorigenic process [59], including genes that metabolize xenobiotic agents like cytochrome P450 isozymes (*CYP*), N-acetyltransferases (*NAT*) and glutathione S-transferases (*GST*), oncogenes like Harvey rat sarcoma viral oncogene homolog (*HRAS*), and genes that affect DNA methylation like methylenetetrahydrofolate reductase (*MTHFR*). For several of these genes about 1.5-fold differences in relative risk have been observed between individuals that carry susceptible *versus* non-susceptible alleles. The influence of low-penetrance genes on familial aggregation of common cancers in humans has recently been reviewed by Houlston and Peto [37].

2.3. Mouse models for human cancer

Genetic and environmental heterogeneity of the human population prevents identification of low-pene-

trance TSGs in an unbiased manner, by linkage analysis. These limitations can be overcome by making use of inbred strains of mice as a tool to study the genetics of complex human diseases. Genetically, inbred strains of mice are completely homozygous and therefore carry only one allele of each gene. Within one inbred strain all individual mice are identical to each other like identical twins, while unrelated inbred strains differ from each other like unrelated individuals. In this way studies of cancer genetics using inbred strains of mice allow comparison of 'tens of a kind' or even 'hundreds of a kind'. This is of particular importance when low-penetrance TSGs are studied that do not exhibit full penetrance towards a disease phenotype. Because mice are maintained under standard conditions, including a standard diet and pathogen-controlled housing, also environmental heterogeneity within each experiment is limited. Importantly, unrelated inbred strains of mice exhibit large variation in susceptibility to various types of cancer, differences that must be caused by allelic variation in TSGs as present in the germline [79]. For practical reasons the tumorigenic process is usually accelerated, either by inducing tumors using carcinogenic agents or by making use of transgenic or (conditional) knockout mice that mimic human cancer [40]. Because of high homology in genomic organization and gene function between mouse and man [4,82], it is expected that many TSGs identified in mouse will have comparable function in humans [45]. Therefore, inbred strains of mice provide an excellent tool to map and identify low-penetrance TSGs with intermediate or even small effects on tumorigenesis.

Studies that aim to map TSGs onto the mouse genome frequently share a common design. In brief, two 'parental' inbred strains of mice are selected that are known to exhibit considerable phenotypic differences in their susceptibility to the type of cancer of interest. These parental strains are used to produce segregating crosses, consisting of hundreds of backcross or F2-hybrid mice. For each hybrid mouse both genetic composition and tumor susceptibility are analyzed. Genetic composition is determined using markers that are polymorphic between the parental strains, e.g. PCR-typable simple sequence length polymorphic markers or SNPs that are equally distributed over the genome [21,60]. Phenotypically, the load, number, size and/or morphology of tumors are examined. Finally, statistical evaluation will reveal what genomic segments are linked to variation in tumor susceptibility. By now, more than 50 TSGs for various types of cancer have been mapped onto the mouse genome using this 'classical' straightforward genetics approach [18, 22]. These results confirm that genetic predisposition to sporadic cancer is indeed influenced by *many* genetic factors.

2.4. Networks of epistatic interactions

Compared to the very strong effects of single genes that dominate predisposition to hereditary cancer syndromes, the effects of TSGs that affect sporadic cancer appear to be modest. Nevertheless, several mouse linkage analysis studies reported the mapping of one TSG with 'major' effects, indicating that one locus explained about half of the variation in cancer susceptibility between two parental strains while the remaining unexplained variation was caused by multiple unmapped TSGs with 'minor' effects [20,32]. These data suggest that the majority of phenotypic variation in cancer susceptibility between two unrelated inbred strains is caused by genetic variation in only two to five TSGs, an estimation that is based on the assumption that the effects of individual TSGs are additive [25]. However, because these studies do not account for putative effects of epistatic interactions, i.e. the possibility that the effect of one TSG is highly dependent on the genotype of a second TSG, the total number of TSGs that contribute significantly to phenotypic variation between two parental strains may be highly underestimated. Systematic examination of epistatic interactions between TSGs in a whole-genome cross is hampered due to statistical limitations. For example, the genotype of hybrid mice must be determined by at least 50 to preferably 100 markers, resulting in a total of 50 to 100 statistical tests to evaluate 'main-effects' of individual markers on tumor susceptibility. In addition, systematic evaluation of the effects of all putative interactions between pairs of TSGs based on genotyping of only 50 markers, i.e. coverage of the genome with just one marker per 30 cM, results in a total of 1225 'pairwise interactions' that need to be incorporated in the statistical analysis ($49+48+47+\cdots+1 = 1225$). On the one hand, this exponential increase in the number of possibilities under investigation increases the statistically required correction factor that has to be applied in order to reduce the number of 'false positive' linkage data. On the other hand, when too many (pairs of) TSGs segregate in such crosses, identification of their individual 'main-effects' and 'pairwise-interaction effects' becomes very complicated.

Systematic analysis of epistatic interactions is facilitated by making use of inbred strains of mice in

which genetic complexity is further reduced. One system in which this was achieved are the Recombinant Congenic (RC) Strains, a tool that was specifically developed to dissect multigenic traits [19]. Series of RC strains consist of about 20 inbred strains that are composed of genetic material derived from two unrelated parental strains, a common 'background strain' and a common 'donor strain'. Each RC strain inherited a random proportion of about 87.5% of its genes from the background strain and only 12.5% of its genes from the donor strain, so on average each RC strain will differ from its background strain in only 1/8th of the total number of TSGs in which both parental strains differ from each other. Extensive analysis of genetic composition revealed that the 12.5% donor-derived genes are distributed among a median of 9 (ranging from 4 to 13) chromosomes [52,72], indicating that crosses between one RC strain and its common background strain can be genotyped with a limited number of markers. In case of ten markers that cover ten independently segregating genomic segments, statistical evaluation requires analysis of 10 'main-effects' and 45 'pairwise interactions' ($9+8+7+\cdots+1=45$). This reduction in genetic complexity was sufficient to allow statistical analysis of all putative pairwise interactions.

The system of RC strains was applied to investigate susceptibility to lung cancer and susceptibility to colon cancer. In addition to analysis of 'main-effects' of individual markers, a systematic search for 'pairwise interactions' was included in the statistical evaluation. These studies resulted in the mapping of 30 TSGs for susceptibility to lung cancer, *Sluc1–Sluc30* [26,28,76], and 15 TSGs for susceptibility to colon cancer, *Scc1–Scc15* [51,65,80,81]. First of all, these data clearly demonstrated that the total number of TSGs in which two parental strains may differ from each other is much larger than the former estimates of two to five TSGs. Moreover, the far majority of these TSGs was shown to participate in one or several pairwise genetic interactions, i.e. 29 out of 30 *Sluc* loci and 11 out of 15 *Scc* loci. Different patterns of interactions were observed, as exemplified in Fig. 1. For instance, allelic variation of *Sluc5* results in large differences in tumor susceptibility in the subset of mice that were homozygous for the 'b'-allele of *Sluc12*, while little effect of *Sluc5* was observed in the subset of mice that were homozygous for the 'o'-allele of *Sluc12* (Fig. 1B). In this example, the maximal 'main effect' of *Sluc5* is underestimated when genetic context is not taken into account (Fig. 1A). Other interactions showed even 'counteracting effects'. For instance, mice that were homozygous for the 'b'-allele of *Sluc8* were more susceptible than mice that were homozygous for the 'o'-allele, at least in the subset of mice that were homozygous for the 'b'-allele of *Sluc6*. In contrast, the 'o'-allele of *Sluc8* was the susceptible allele in the subset of mice that carried the 'o'-allele of *Sluc6* (Fig. 1D). In this example, neither *Sluc6* nor *Sluc8* would be detected when genetic context is not taken into account, because in this cross their net effect on tumor susceptibility is about zero (Fig. 1C). Therefore, despite the large number of TSGs that were mapped, these data illustrate that *each* TSG can have large main-effects. However, these main-effects are frequently masked due to their involvement in one or several epistatic interactions, and their maximal effect can only be visualized once they are analyzed on a permissive genetic background.

The total number of TSGs that affect just one particular type of cancer may be quite large. For example, as many as 30 *Sluc* lung cancer loci were revealed by linkage analysis of segregating crosses between various RC strains and their common background strain that together covered only half of the mouse genome [76]. Extrapolating this result, analysis of the other half of the genome might reveal another 30 TSGs. In addition, any given combination of two common inbred strains of mice will not be polymorphic in all TSGs, so other combinations of inbred strains will reveal even more new TSGs. Based on these assumptions it is estimated that susceptibility to one type of cancer is influenced by more than 100 TSGs, most of which are involved in one or more epistatic interactions. Due to statistical limitations the studies using RC strains were restricted to the systematic analysis of 'pairwise' interactions. However, the observation that several TSGs were shown to interact with multiple partners suggests that higher order interactions do exist, and indicate that TSGs form large networks of gene interactions that are underlying genetic predisposition to cancer.

3. TSG candidate genes

Genes encode functional units in an organism. Hence, genetic networks must represent molecular interactions like protein–protein interactions, or indirect interactions between molecular pathways that influence each others function. This raises the following questions: What kind of molecular networks are represented by genetic networks? And how do these networks affect predisposition to cancer? To address these questions it will be necessary to actually identify

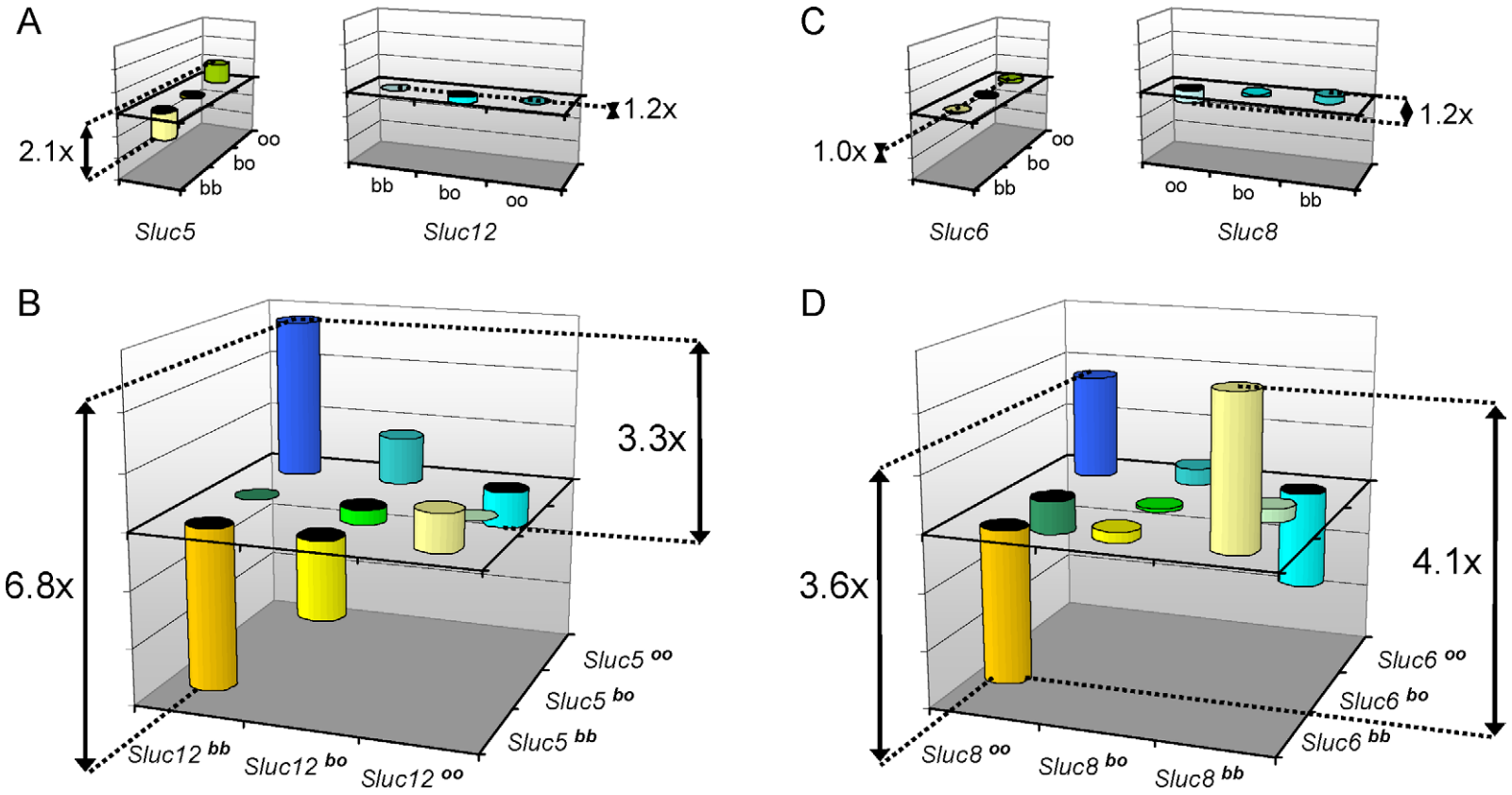

Fig. 1. Main-effects of TSGs are masked by their involvement in epistatic interactions. Graphic representation of estimated main-effects (A and C) and interaction effects (B and D) of lung cancer susceptibility loci *Sluc5* and *Sluc12* (A and B), and *Sluc6* and *Sluc8* (C and D), based on their original normalized mapping data [28]. The cylinders indicate magnitude and direction of deviation from the mean (horizontal planes). Genotypes of F2-hybrid mice are indicated by: 'bb', homozygosity for the b-allele; 'bo', heterozygosity; and 'oo', homozygosity for o-allele. A: The observed main-effect of genetic variation in the *Sluc5* locus on lung tumor size (2.1-fold difference) is underestimated when genetic interactions are not taken into account. B: The main-effect of *Sluc5* is large within the subset of mice that carry the $Sluc12^{bb}$-genotype (6.8-fold difference), and relatively small in mice that carry the $Sluc12^{oo}$-genotype. The *Sluc12* locus also affects lung tumor size (up to 3.3-fold difference), in a *Sluc5*-dependent manner. C: The main-effects of *Sluc6* and *Sluc8* on lung tumor number are completely annihilated by their involvement in a counteracting interaction. D: Both *Sluc6* and *Sluc8* cause significant differences in tumor number (up to 3.6-fold and 4.1-fold, respectively); however, the direction of their effects depends on the genotype of their interacting partner.

TSGs. In contrast to the large number of more than 100 susceptibility genes for various types of cancer that have been mapped onto the mouse genome, only few TSGs have been cloned and even less have been characterized to the extent that we understand their molecular function in tumor development. On the one hand, the efforts that are required to identify a TSG by positional cloning procedures are huge. Fine mapping of a TSG requires production of congenic and recombinant strains that all need to be examined for their cancer susceptibility phenotype. Once the genomic segment that contains the TSG is small enough to examine all putative candidate genes, it may be quite difficult to predict what kind of experiments need to be done to obtain ultimate proof that a candidate gene truly represents a TSG [18]. On the other hand, it may not be necessary to identify all TSGs by positional cloning procedures. As soon as one or few TSGs pinpoint a molecular mechanism that affects susceptibility to cancer, clues about the function of its interacting partners will facilitate their identification.

3.1. Genes that function cell-autonomously

Tumorigenesis is characterized by accumulation of somatic mutations in oncogenes and tumor suppressor genes, resulting in clonal expansion of tumor cells that gain enhanced proliferative capacity, that loose sensitivity to apoptotic signals, or acquire other selective advantages. Because clonal expansion results from selective advantage that was acquired by those cells that gained somatic mutations, oncogenes and tumor suppressor genes must function cell-autonomously. This group of genes forms an excellent group of candidate TSGs, as polymorphisms that modify their function are likely to affect the rate at which the process of tumorigenesis takes place. It is interesting to note that genes that are causally responsible for highly penetrant hereditary cancer syndromes frequently belong to this category of cell-autonomously functioning genes, and that their extreme effects on cancer risk resemble a situation in which the first 'hit' in the multistep process of tumor development has already been made before birth. However, many polymorphisms in cell-autonomously acting genes will affect gene function less dramatically, leading to the expectation that there is a whole spectrum of allelic variants with more subtle effects. Although these moderate effects do not result in monogenic inheritance of cancer within families, they will contribute to the overall genetic predisposition to cancer of an individual. One example of an oncogene that has been identified as a TSG for lung cancer is the mouse *Kras2* gene. Due to a polymorphism in an intronic sequence that affects *Kras2* expression levels, somatic mutations in the relatively highly expressed *Kras2* allele are more tumorigenic than somatic mutations in the lowly expressed *Kras2* allele [11,12], resulting in more and larger lung tumors [29,30]. Another example is *Ptprj*, which was identified as the candidate gene for the colon cancer susceptibility locus *Scc1* and behaves as a tumor suppressor gene in human colorectal cancer [64,66]. Recently, polymorphisms in *PTPRJ* were associated with breast cancer risk in humans [45]. These examples demonstrate that polymorphisms in oncogenes and tumor suppressor genes with moderate effects do influence cancer susceptibility.

3.2. Genes that function systemically

Although neoplastic cells loose responsiveness to certain signals from their surroundings, tumor development is not a process that takes place independently from its microenvironment. Interactions between neoplastic cells and their neighbouring stromal cells like macrophages, mast cells, fibroblasts, and endothelial cells play a critical role in tumorigenesis by mediating processes like proliferation, adaptive immunity, angiogenesis and metastasis [7,15]. Therefore, in addition to cell-autonomously functioning oncogenes and tumor suppressor genes, candidate genes for TSGs also include allelic variants of systemically acting genes that modulate tumorigenesis by influencing tumor microenvironment. The group IIA *secretory* phospholipase A_2(PLA_2) gene *Pla2g2a* was identified as the candidate gene for *Mom1*, a modifier of Apc^{Min}-induced intestinal neoplasia that represents a mouse model for human FAP [13,47]. Despite serious efforts to identify somatic mutations in *Pla2g2a* in sporadic cancer no such mutations were detected, indicating that *Pla2g2a* is not an oncogene or tumor suppressor gene [63]. Therefore, due to its paracrine action *Pla2g2a* is the first example of a TSG that influences tumor susceptibility by modulating tumor microenvironment.

3.3. A network of inflammatory genes

PLA_2 enzymes play an important role in mediating inflammatory responses by releasing arachidonic acid and other fatty acids from membrane phospholipids. Arachidonic acid can be further metabolized into mediators of inflammation like prostaglandins and

leukotrienes via the action of cyclooxygenases (COX) and lipoxygenases, respectively. Interestingly, knockout mice for cytosolic PLA_2, COX-1, COX-2, and the prostaglandin E_2 (PGE_2) receptors EP2 and EP4, were all shown to affect susceptibility to intestinal neoplasia [36,44,54,71]. Moreover, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the production of prostaglandins *and* reduce the number of Apc^{Min}-induced intestinal tumors [38,39], whereas in humans NSAIDs have been shown to reduce the number of colorectal tumors [5,67]. Other examples of links between inflammatory genes and tumor susceptibility include the observations that mice deficient for the anti-inflammatory cytokine interleukin-10 (IL-10) suffer from chronic intestinal inflammation *and* exhibit a high incidence of colorectal cancer [6], while mice deficient for the pro-inflammatory cytokine tumor necrosis factor α (TNFα) have a reduced incidence of skin tumors [53]. Knockout mice that lack the enzyme inducible nitric oxide synthase (iNOS), which is responsible for the production of nitric oxide (NO) during inflammatory responses, exhibited an *increased* incidence of intestinal tumors [70] and a *decreased* incidence of lung tumors [43], demonstrating that the "knockout allele" as well as the "wild-type allele" could both prevent and promote tumorigenesis. This example illustrates how identification of one TSG, i.e. identification of *Pla2g2a* as a TSG for intestinal cancer, pinpoints the molecular basis of inflammation to genetic predisposition to cancer.

By now there is ample evidence that many tumor-promoting signals are provided by hematopoietic inflammatory cells [16]. Proof that genetic variation in hematopoietic cells influences susceptibility to tumors of epithelial origin was provided by elegant experiments in which skin tumorigenesis was followed upon bone marrow transplantations using genetic variants of the matrix metalloproteinase MMP-9 [14]. These data clearly demonstrated that genes that mediate inflammatory responses are excellent candidate genes for TSGs, supporting the notion that the molecular basis of inflammation forms a network of interacting TSGs that affects tumorigenesis systemically through modulation of tumor microenvironment.

4. Determination of genetic predisposition to sporadic cancer

Initiation and progression of cancer is influenced by cell-autonomously acting processes in neoplastic cells like proliferation, differentiation, and apoptosis, as well as by systemically acting processes that are mediated by stromal cells within tumor microenvironment like angiogenesis, immune responses, and tissue remodeling. In principle, all genes that modify any of these biological modules are candidate genes for TSGs, as polymorphisms in these genes can affect tumor development. Therefore, one approach to estimate an individual's genetic predisposition to cancer is by determination of the genotype of TSGs. Alternatively, new methods are being developed that aim to characterize 'phenotypic representations' of defined biological modules that can be associated with cancer risk.

4.1. SNP genotyping

The effects of cancer-associated alleles of genes like *APC* and *BRCA1* (breast cancer 1, early onset) are very strong, exceeding by far the miscellaneous effects of all other hereditary and environmental factors. Due to the very high penetrance of these high-risk alleles towards a cancer phenotype, determination of their genotype is a useful approach to predict whether an individual is at increased risk for cancer or not. Besides high-penetrance TSGs, a whole spectrum of intermediate- and low-penetrance TSGs may contribute to the relative risk for cancer. As indicated in Section 2.2, identification of low-penetrance TSGs from the human population by linkage analysis has proven to be difficult, while association studies revealed some effects of polymorphisms in typical candidate genes that are likely to influence tumorigenesis. By now, large scale genome-wide SNP genotyping using microarray-based SNP-chips is becoming feasible, raising the expectation that numerous polymorphisms will be identified that are associated with complex diseases like cancer [73]. These technological developments will allow to estimate relative risk roughly by summing up 'main-effects' of low-penetrance TSGs. However, evaluation of genetic predisposition by TSG genotyping will be restricted to those TSGs that exhibit a 'main-effect' by being composed of alleles that are consistently associated with either increased or decreased risk for cancer. Hence, genotyping will not be suited to determine genetic predisposition caused by interacting TSGs whose individual alleles may both confer to 'susceptibility' and 'resistance', depending on genetic background (Fig. 1).

4.2. Phenotypic representation of TSG networks

The common existence of extensive genetic interaction networks was recently demonstrated in yeast [35,74]. Studies of mouse models for human cancer demonstrated that a significant proportion of genetic predisposition is influenced by interacting TSGs (see Section 2.3). As a consequence, knowledge about the genotype of one or few TSGs that participate in a network of interactions will be insufficient to predict relative risk to sporadic cancer. Even when the genotypes of all interacting partners are known it will be a challenging task to predict their effect, because networks of gene interactions tend to buffer genetic variation [34]. Therefore, instead of predicting cancer risk by SNP-genotyping, an alternative approach should be applied that reveals the net phenotypic effects of TSG networks on cancer-associated biological modules. Systemically acting TSGs affect cancer risk indirectly as a consequence of their primary effects on tumor microenvironment. New strategies aim to analyse the 'indirect' effects of TSG networks on tumor susceptibility by examination of their 'direct' effects on cancer-associated biological modules, and to associate these data with cancer risk. Two examples of such developments will be discussed in more detail, one concerning analysis of the macrophage inflammatory response and the other concerning the fibroblast wound-response.

4.2.1. Macrophage-associated inflammatory response

Identification of one or few TSGs will be sufficient to pinpoint cancer-associated biological modules, as exemplified by identification of *Pla2g2a* that linked the molecular network of inflammation to tumor development (see Section 3.3). Macrophages are hematopoietic inflammatory cells that are known to mediate inflammatory responses *and* tumorigenesis. They belong to the mononuclear phagocyte system, a population of cells derived from progenitor cells in the bone marrow that differentiate into monocytes, circulate in the blood, and then enter tissues where they adapt to their local environment to become resident tissue macrophages. Interestingly, macrophages exhibit a wide range of phenotypes. On the one hand they function as sensors for pathogens by expressing a variety of pattern recognition receptors that interact with microbial components. Stimulation through these receptors is sufficient to initiate inflammatory responses [2,8]. On the other hand macrophages can recognize altered-self molecules, for instance as present on apoptotic cells, and actively suppress inflammatory responses [3,69]. This phenotypic plasticity can be demonstrated *in vitro* by exposing macrophages to different challenges like microbial stimuli, immunoglobulin-opsonized particles, cytokines, and apoptotic cells [33]. Importantly, different types of macrophage activation can contribute both positively and negatively to different stages of tumorigenesis by influencing processes like angiogenesis, tissue remodeling, and subversion of anti-tumor immunity [48,49,61,78]. Hence, allelic variation in genes that affect macrophage inflammation-related characteristics are likely to affect tumor development.

Linkage studies resulted in the mapping of 'macrophage-associated risk inflammatory factors', *Marif* loci, by analysis of a panel of inflammation-related assays that was applied to cultures of primary bone marrow-derived macrophages from F2-hybrid mice [31]. *Marif1* and *Marif2* each affected expression of iNOS, secretion of TNFα, and secretion of IL-12, genes that are known to modulate processes like angiogenesis, adaptive immune responses, and tumorigenesis. Interestingly, *Marif1* and *Marif2* were tightly linked to the lung cancer susceptibility loci *Sluc6* and *Sluc20*, respectively [28,76], as well as to a pair of interacting loci that determine lung tumor shape, *Ltsd4* and *Ltsd3*, respectively [75]. *Marif1* was also linked to an intestinal cancer susceptibility locus, *ssic1* [27], while *Marif1* and *Marif2* colocalized with the 'cytokine induced activation' loci *Cinda3* and *Cinda5*, respectively, which affect proliferation of T-cells upon stimulation with IL-2 or IL-4 [46]. Importantly, all these loci were mapped using the same combination of parental strains, suggesting that the cluster of *Marif1* / *Cinda3* / *ssic1* / *Sluc6* / *Ltsd4* susceptibility loci on the distal part of mouse chromosome 4 and the cluster of *Marif2* / *Cinda5* / *Sluc20* / *Ltsd3* susceptibility loci on the proximal part of chromosome 8, each consist of one gene that affects both quantitative and qualitative aspects of tumorigenesis by modulation of tumor microenvironment. Identification of these genes and characterization of their function will help to unravel the complex relationship between innate immune responses, adaptive immune responses, tumor initiation and tumor progression. In principle, such knowledge can be applied in humans to link the characteristics of an individuals inflammatory response to this individuals cancer risk, for instance by analysis of a panel of inflammation-related assays using blood-derived leukocytes. Alternatively, the genetic makeup of inflammatory cells is also reflected by the profile of genes and proteins that are expressed by these cells.

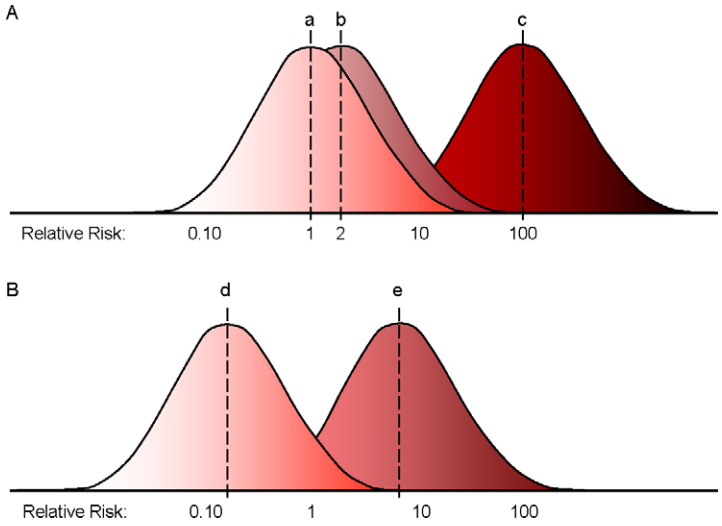

Fig. 2. Relative distribution of populations at risk for familial and sporadic cancer. A: The relative risk for the general population ('population a') to get cancer is 1. Determination of genetic predisposition to cancer by genotyping of intermediate- and high-penetrance TSGs will reveal (small) subpopulations of people at intermediate ('population b') and high ('population c') risk for cancer. B: The general population harbors relatively large subpopulations of people at low risk ('population d') and high risk ('population e') for sporadic cancer. New methods are being developed to identify these populations, by characterization of cancer-associated biological modules that reveal the net effect of networks of low-penetrance TSGs.

Genomics and proteomics approaches allow to characterize RNA and protein profiles, respectively, using technologies that are more suited for large-scale automated handling of samples than analysis of *in vitro* cultures of primary cells [68,83,84]. In this way, RNA or protein expression analysis of blood-derived leukocytes provides a phenotypic representation of TSG networks that affect development of tumors of epithelial origin through their function in hematopoietic inflammatory cells.

4.2.2. Fibroblast-associated wound response

Fibroblasts are another example of non-epithelial stromal cells that modulate microenvironment of tumors of epithelial origin [7]. Based on the observation that tumors share quite some characteristics with 'wounds that do not heal' [23], it was hypothesized that *in vitro* cultures of serum-activated fibroblasts might resemble stromal fibroblasts in tumor microenvironment. First, RNA expression patterns of genes in non-activated primary cultures of fibroblasts were compared to those of serum-activated fibroblasts, revealing a profile consisting of genes that allowed to discriminate 'normal' from 'wounded' cells. Next, this fibroblast wound-response signature was used for cluster analysis of various tumor types, and was shown to be an excellent predictor for the clinical course of cancer progression in humans [9,10]. These data illustrate that phenotypic representation of a tumor-related molecular process that is mediated by non-tumor cells can be used to predict tumor progression, and suggests that fibroblast-derived profiles can be applied as a representation of a TSG network that affects tumorigenesis through modulation of wound-response.

5. Future perspectives

Predisposition to hereditary cancer syndromes is dominated by the strong effects of high-penetrance TSGs, while predisposition to sporadic cancer is strongly influenced by the combination of multiple low-penetrance TSGs. Genetic predisposition to familial cancer can be estimated by analysis of the genotype of high- and intermediate-penetrance TSGs, because the contribution of each allele of these TSGs to cancer risk is more or less independent from genetic background (Fig. 2A). In contrast, analysis of the genotype of low-penetrance TSGs has little predictive value for estimation of cancer risk, in particular when their individual effects are highly dependent on genetic background due to their involvement in genetic interactions. Therefore, SNP-genotyping and other methods that are currently used to estimate familial cancer risk are not suited to estimate an individuals risk for sporadic cancer. Recent insights into the function of TSG-networks emphasize the need to evaluate cancer risk by deter-

mination of some kind of phenotypic representation of cancer-associated biological modules (Fig. 2B). One advantage of such an approach is that it does not matter whether genetic variation in cancer risk is caused by one, ten, or fifty TSGs, because the net outcome of the combined action of all TSGs that influence one particular biological module is being determined. Another advantage of this approach is that it reveals functional information about cancer-associated biological modules, information that can be used to determine what strategy will be most effective to prevent or treat cancer in an individual.

Several hurdles still need to be taken before an individuals risk for sporadic cancer can be determined. First, the biological modules that are relevant to tumor initiation and tumor progression need to be pinpointed. This can be achieved by identification of a limited number of interacting low-penetrance TSGs using mouse models of human cancer. Next, these biological modules need to be characterized phenotypically, preferably by using *in vitro* model systems in which they can be analyzed separately from other potentially interfering biological processes. Although there are many ways in which a phenotypic representation of an isolated biological module could be obtained, determination of RNA-expression profiles of cells that play a crucial role in the biological module of interest currently seems to be the most feasible approach, in particular because microarray expression analysis is becoming common practice in many laboratories and is a suitable technique for high-throughput screening. Moreover, this technique may be applied in clinical practice in the near future to estimate the risk of cancer progression, in order to decide what therapy will be most useful to the patient [1,77]. Finally, each phenotypic representation needs to be validated and associated with cancer risk. Hence, the major challenge for genetic research within the next decade is to establish 'signatures' that reflect phenotypic representation of genetic variation in networks of low-penetrance TSGs in order to estimate an individuals risk for sporadic cancer. These developments will offer new opportunities to improve population-wide cancer screening programmes, and to intervene in tumor initiation and tumor progression in a patient-tailored manner.

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