Chapter 6

Summarizing Discussion
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The genetic contribution to the development of cancer is obvious in familial cancer syndromes that are due to germ-line mutations in tumor suppressor genes, like APC or mismatch repair genes, such as MSH2. These syndromes account for only a small portion of all cancer cases, while the non-familial, sporadic, type of cancer with no apparent genetic contribution represents the majority of cases. However, the genetic predisposition, controlled by numerous low penetrance susceptibility genes with relative small effects (1), contributes to a large proportion to the sporadic cancers (2). Unfortunately, family and population studies do not have sufficient resolving power to identify the relevant genes involved in the development of non-familial cancer, even in the case of genes with sizeable effects. Therefore, mouse models with the advantage of defined genetic composition, the possibility of producing informative crosses, and standardized tumor induction protocols, are being used to resolve the genetics of cancer susceptibility (Chapter 1). Subsequently, the human homologues can be identified exploiting the well-defined homologies between mouse and human genomes.

For mapping studies, we used the recombinant congenic mouse strains (RCS), which are specifically designed to identify individual genes involved in the control of multigenic traits, such as cancer (3, 4). In a series of 20 homozygous RCS each strain carries a different random portion of 12.5% of genes of one inbred strain (donor strain) on the genetic background of the second inbred strain (background strain). In this way, 20 different random combinations of genes of the donor strain and the background strain are represented. A cross between a susceptible RCS and the background strain are used for mapping experiments. Consequently, in such a RCS cross the number of segregating genes (i.e. genetic heterogeneity) is reduced, and hence the mapping power is increased. The CcS-strains of RCS, which are derived from the colon cancer susceptible strain STS and the resistant background strain BALB/c, are used to map genes controlling colon cancer development. Previously, five susceptibility loci to colon cancer, Scc1-Scc5, have been mapped using susceptible RCS CcS-19 (5, 6). All five loci were confirmed in an independent identical cross increasing the confidence of mapping (Chapter 3). In addition, we have mapped nine new colon cancer susceptibility loci: Scc6-Scc9 using the RCS CcS-3, CcS-5 and CcS-11 (Chapter 2) and Scc11-Scc15 using RCS CcS-19 (Chapter 3). For all novel Scc loci, recombinant haplotypes need to be generated and tested for colon cancer susceptibility with the aim of further reducing and fine-mapping the candidate regions. When the loci are mapped to relative small genomic segments, the available mouse and human genome sequence and new array expression and SNP (single nucleotide polymorphism) technologies will facilitate the detection of candidate genes.

The majority of Scc loci does not operate individually, but modifies each other's action, that is, the Scc alleles are not intrinsically susceptible or resistant, but their phenotype depends on the genotype of the interacting locus. These genetic interactions may offer potential advantages in the
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Identification of candidate genes, since they could operate in the same or in interacting pathways (i.e. the gene products must interact either directly or indirectly). The frequency of genetic interactions is high indicating that it is a common feature in the genetics of cancer susceptibility (6-11). Interestingly, most of these genetic interactions controlling cancer susceptibility are identified using the RCS system: 11 genetic interactions for colon tumor susceptibility (6, 9) (Chapter 3) and 25 interactions for lung tumor susceptibility (7, 8, 11). This observation may be explained by the reduced genetic complexity: given a complex multigenic trait with numerous interacting loci, it allows the systematic testing not only of all main effects, but also of approximately all possible two-way genetic interactions (11). In contrast, for crosses in which the whole genome is segregating, it is unrealizable to systematically screen for all genetic interactions, unless specific selection criteria are applied to study only small subsets of interactions. Nevertheless, some interactions are reported in whole genome crosses (10, 12).

Present efforts to identify cancer susceptibility genes in human populations are limited to association studies involving candidate genes (Chapter 1). The ability to detect significant associations in human populations will be affected by the high frequency of genetic interactions that, depending on 'genetic background', can make a particular locus invisible or more difficult to identify. However, the identification of interacting pathways between susceptibility genes first in the mouse will simplify this search by guiding the choice of candidate genes for association studies in humans (13).

To test possible relationships between susceptibility loci of different types of cancer, the location of the 15 Scuc loci was compared with the location of 30 Sluc (Susceptibility to lung cancer) loci (7, 8, 11) (Chapter 3). Despite the differences in genetic backgrounds and tumor induction protocols used to map the two types of cancer susceptibility loci (see Chapter 3 fig. 1), a significant co-localization between the Scuc and Sluc loci was observed (Chapter 3). This appealing finding supports the idea that susceptibility to several (sporadic) cancer types may utilize the same susceptibility alleles and/or molecular pathways. An alternative explanation recently proposed by analysis of the human genome sequence (14) is the clustering of functionally related genes. In this interpretation, the co-localization of susceptibility loci reflects the association with different genes in the same cluster, rather than one common allele. In fact, susceptibility loci detected as one locus may be multiplets containing several individual genes, each of which has a small effect. Direct evidence in favor of this idea has been provided by the detection of at least two genes in the Mom-1 locus (15) and the Scell locus (Chapter 4), and of similar results obtained from susceptibility to other diseases, such as systemic lupus erythematosus in which the locus Sle1 is split into four separate susceptibility loci (16). Although the complete understanding must await future identification of the relevant cancer susceptibility genes, it is evident that knowledge of shared genes or identical pathways for several (sporadic) cancer types may unravel the mechanism by which cancer susceptibility operates and additionally will provide information for possible therapeutic interventions.
One of the Scc loci, Scc1, has been limited by recombinant mapping to an interval of less than 300 kb containing the gene protein-tyrosine phosphatase receptor type-J (Ptprj) as the only coding region (Chapter 4). The susceptible STS and resistant BALB/c alleles of Ptprj contain multiple polymorphisms. Some of them result in amino acid substitutions, but none disrupt the open reading frame of the gene (Chapter 4). Perhaps, the molecular and biological differences that account for the susceptible STS phenotype can be defined by functional assays with the BALB/c and STS alleles of Ptprj.

Loss of one copy of PTPRJ in a significant number of human cancers (colon, lung and breast) strengthens the notion that the role of PTPRJ is relevant to cancer susceptibility and additionally, points to PTPRJ as a potential tumor suppressor gene (Chapter 4). Sequencing the exons of PTPRJ revealed 7 polymorphisms of which 5 result in amino acid substitutions. Most of the amino acid substitutions in human as well as in mouse PTPRJ/Ptprj molecules occurred in the exposed regions of the fibronectin type-III (FN-III) domains which are involved in intra- and intermolecular interactions (17, 18). This indicates that they have the potential to modify interactions with ligands or other proteins and thus effect the signaling process. In humans, the polymorphism Gln276Pro (A1176C) exhibits preferential loss of the A-allele in LOH suggesting that the 'resistant' A-allele (Gln) is lost and the 'susceptible' C-allele (Pro) remains unaltered. It is possible that the 'susceptible' C-allele is less functional. This interpretation is supported by two colon tumors from homozygous A1176 patients that show a somatic A1176→C substitution in one of the alleles (Chapter 4). Functional assays, like transformation and colony formation assays, with the different allelic forms of PTPRJ are required to test the further role of this polymorphism in cancer predisposition. In addition, the other polymorphisms in PTPRJ can be tested for allele specific LOH and subsequently in functional assays. Moreover, association studies comparing the PTPRJ polymorphism frequencies of a normal population with those of colorectal patients can be performed to test the possibility that some allelic variants of PTPRJ predispose to cancer. One disadvantage of such studies is that probably thousands of controls and patients need to be tested to detect a significant effect. Another problem might be the definition of the control group.

Since only two somatic mutations in PTPRJ are identified, it is not a classical tumor suppressor. Based on the classical 'two-hit' model of Knudson (19), the presence of inactivating mutations in the remaining PTPRJ allele in tumors with LOH is required as proof for tumor suppressor function. Although the 'two-hit' model has been applied to many familial cancers, it has not proved to be very useful in the definition of the 'new' tumor suppressor genes in sporadic cancers based on LOH (20, 21). In fact, the proportion of the nonclassical tumor suppressor genes is presently considerably underestimated (22). It is possible that loss of one allele of PTPRJ is sufficient to provide selective advantage. The effects of haploinsufficiency have become obvious for many tumor suppressor genes in appropriate knockout mice (p27 (23); p19 (24); Pten (25); Dmp1 (26)). The effect of Ptprj could be tested in knockout mice as well, since one copy of PTPRJ is deleted in human colon tumors. Currently, Ptprj knockout mice are being generated. Alternatively, epigenetic mechanisms might play a role in silencing PTPRJ. No doubt future
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Experiments of gene expression and promoter methylation, and further mutation hunting will provide the answer. In the meantime, there are other questions to answer, how PTPRJ functions as a tumor suppressor. Clearly, much remains to be done to unravel the complexities of this interesting gene and its role in cancer.

Nevertheless, there is evidence that PTPRJ functions consistent with a tumor suppressive activity: a) its transfection into human non-differentiated breast cancer cells induces differentiation and inhibits growth (27), b) its expression in transformed rat thyroid cells suppresses their neoplastic phenotype (28), and c) its overexpression in vascular epithelial cells represses cell cycle progression by inhibiting c-fos and cyclin A promoter activity (29).

To further evaluate the occurrence of PTPRJ loss in cancer, its correlation with other specific chromosomal aberrations was investigated (Chapter 5). A strong correlation between LOH of PTPRJ and loss of chromosomal region 18q12-21 was detected in progressive colorectal adenomas. LOH of PTPRJ seemed to occur earlier than loss of 18q12-21 in adenomas suggesting that a subsequent loss of 18q12-21 contributes to the development of a progressed form of adenomas.

In conclusion, this thesis describes not only the mapping of nine novel Scc loci, but also the identification of Ptpj as the candidate gene for Scc1, the subsequent search for its human homologue, and its role in human cancer. The identification of a large number of cancer susceptibility loci and eventually genes in combination with SNP and array technologies will permit to test the tumor susceptibility profiles of cancer patients. This information may help to reveal the presently unrecognized biological heterogeneity of (non-familial) cancers, with potential implication for prevention or individually optimized treatment strategies.
References