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Review

Tumor Suppressor Genes on Human Chromosome 3 and Cancer Pathogenesis

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Abstract. *The short arm of chromosome 3 is frequently altered in human cancers of different tissue origin. Certain regions on the chromosome arm 3p have been defined by deletion studies in human cancer cells and tissues. Also, regions at 3p are eliminated in microcell hybrids parallel to increased tumorigenicity in immunosuppressed mice. We have analysed chromosome instability and several genes involved in tumor pathogenesis at 3p, such as VHL, RIITGFB, CATNB, MLH1 and FHIT. By studying eleven tumor types, we have shown that the importance of CER1 (common eliminated region 1) transgresses tissue specificity. Comparative studies on losses of VHL, FHIT/FRA3B and CER1 show that the CER1 region is preferably lost in human tumors. Alterations of FHIT are associated with reduced survival of breast and colon cancer patients. The FHIT gene is located at the constitutive fragile region, FRA3B. Chromosome 3, particularly FHIT, is unstable in breast cancer patients who have germ line mutation in the BRCA2 gene. The chromosome instability in BRCA2 tumors reflects the DNA repair mechanism of the gene product Brca2. It can be concluded that our results reflect a synergism of tumor suppressor gene (TSG) losses at the chromosome 3p region in relation to the biological behavior of tumor cells and tumor pathogenesis.*

When technological developments in karyotyping allowed analysis of chromosome alterations in solid tumors, it was observed that chromosome 3 was frequently altered. These findings were followed up by molecular methods, showing deletions at the short arm of chromosome 3 in lung and kidney tumors (1, 2). Today, loss of the short arm of chromosome 3 is considered to be among the most frequent genomic changes in human solid tumors. In addition to lung and renal cancer, these include mammary, nasopharyngeal, ovarian, testicular, cervical, head and neck and other cancers (3, 4).

Genetic alterations at 3p have been shown to be associated with growth progression and the clinicopathological characteristics of tumors. We have shown that deletions at 3p in breast cancer are associated with elevated aneuploidy and S-phase and reduced patient survival (3). A variable frequency of deletions at 3p is detected in different tumor types (5). The deletion frequency at 3p is even different in tumors within the same tissue; we have described it as higher in ductal than in lobular breast cancer (6). Extensive studies have not been able to pinpoint a "strong" tumor suppressor gene (TSG) involved in multiple primary malignancies. In a way, this can be considered as a drawback of the ongoing studies, but is also of interest with regard to the synergism of TSGs losses in tumor pathogenesis.

Until recently, only a few TSGs had been identified at 3p, but this number has been increasing and today the candidate TSGs number more than a dozen (Figure 1). Some of the TSGs are well-characterized with respect to tumor suppressor function, while fewer data are available for the more recently identified genes. Several regions on 3p harbor TSGs and are commonly deleted in tumors. One of these regions is the 3p25.3 region, which contains the von Hippel-Lindau (VHL) TSG, and another is the 3p14.2 region, which contains the FHIT TSG.

VHL and MLH1 are well-characterized TSGs at 3p

The VHL at 3p25.3 is a well-characterized TSG with respect to biological function in relation to malignant phenotype. The Vhl is a key negative regulator of angiogenesis, that is considered to be a rate-limiting step in tumor progression. Its protein product is responsible for the degradation of the alpha subunit of the HIF transcription factor in the ubiquitin-proteasome pathway (7). The HIF regulates several genes important for angiogenesis. Loss of VHL

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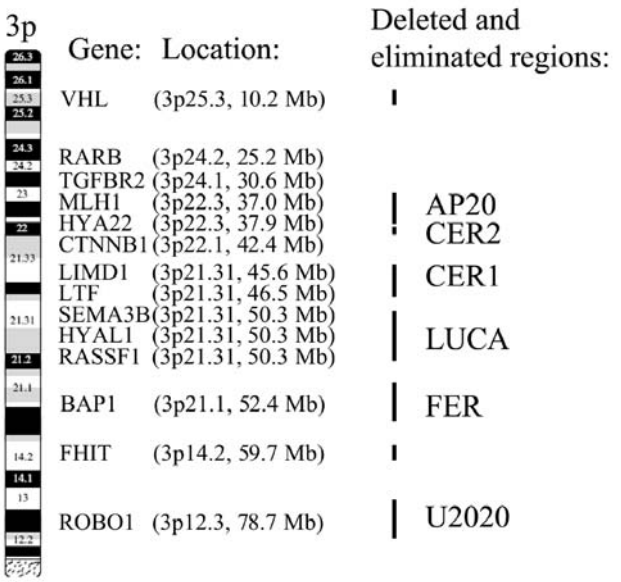

Figure 1. *TSGs and putative TSGs at chromosome arm 3p. Information in parentheses is based on the chromosome band and on megabases (Mb) from 3p telomere on the physical map (http://www.ensembl.org/). Vertical lines indicate the approximate location of frequently deleted or eliminated regions. The lines are not exactly to scale.*

function in tumors results in increased angiogenesis in several malignancies. A clear example of VHL deactivation in relation to tumor pathogenesis is the detection of mutations in kidney tumors, hemangioblastoma and pheochromocytomas (8). Inactivation of the VHL gene is a prevalent genetic alteration in both hereditary and sporadic renal carcinomas. Frequent 3p deletions including the VHL region have been reported in multiple malignancies, including renal cell, ovarian and endometrial carcinomas.

While the VHL can be considered as a gatekeeper TSG, the MLH1 at 3p22.3 is a caretaker TSG. Its protein product is involved in DNA mismatch repair. Germ-line mutations are associated with the HNPCC cancer syndrome and inactivation, particularly by epigenetic mechanism, occurs at somatic level (9).

Additional relatively well-defined TSGs at chromosome arm 3p are the RIITGFB, (encoding the TGF beta receptor II) involved in anti-growth control and the CATNB (encoding beta-catenin), involved in cell adhesion and transcriptional control. As for MLH1, alterations in RIITGFB and CATNB are of interest in relation to several malignancies, particularly those of gastrointestinal origin (10, 11). By analyzing the effect of mismatch repair deficiency on tumor pathogenesis, we have shown that genes containing short repeated sequences are preferably mutated, including the RIITGFB gene (11).

FHIT, BRCA2 and chromosome instability

FHIT is a large gene at 3p14.2, which spans one of the most active common fragile sites of the human genome, the aphidicolin-sensitive site FRA3B. The Fhit protein has diadenosine triphosphate hydrolase activity. It is not clear if the hydrolase activity is associated with the tumor suppressor function. Although point mutations are rare, several studies support a tumor suppressor function of Fhit, including deactivation in human tumors and in knockout mice (12). Fhit activity is associated with cellular pathways that relate to tumor pathogenesis, including apoptosis and cell cycle arrest, where p53 pathways are involved (13, 14).

Loss of FHIT is evident in various tumors and cell lines, as well as abnormal RNA expression and/or absence of protein (15, 16). We investigated the deletion frequency at the FHIT locus in ten tumor types and found deletions in half the tumors, ranging from 30% to 66%, depending on tumor location (16, 17). These data indicated that deletion of the FHIT gene is a fairly common event in all the tumor types analyzed. Also, an epigenic alteration in the form of methylation is an alternative mechanism for silencing FHIT (18). Deletions of FHIT have been shown to be an indicator of poor prognosis in several tumor types, including breast, colorectal, lung and stomach cancer, as shown by association with reduced patient survival (16, 17).

Support for the role of FHIT as a tumor suppressor comes from FHIT gene replacement and knockout experiments. Stable exogenous FHIT expression in FHIT-negative cancer cells resulted in inhibition of tumor cell growth in immunosuppressed mice (19). Gene knockout studies showed that both homo- and heterozygous negative FHIT mice have a higher frequency of developing spontaneous and carcinogen-induced tumors, as compared to normal FHIT +/+ littermates (20). There was no difference in frequency of either spontaneous or carcinogen-induced tumor development in FHIT +/– and FHIT –/– mice, suggesting that haploid insufficiency of FHIT may promote tumor growth. This would explain the similar pattern of tumor spectra observed in mice with one or both FHIT alleles inactivated, and the general lack of somatic point mutations and germ-line mutations in FHIT-associated cancers.

Genome instability is high in some hereditary breast cancer, particularly in tumors of BRCA1 and BRCA2 mutation carriers, in line with the role of the gene products in DNA repair (21, 22). In familial breast cancer kindreds, early studies by us showed a high frequency of deletions at 3p14 relative to the frequency of deletions in the same region in sporadic breast cancer (23). In follow-up studies, we described a relatively high frequency of alterations at FHIT in sporadic tumors, and a much higher frequency in BRCA2-mutated tumors (24, 25). This could merely reflect the unstable nature

Table I. *Tumor suppressor genes at 3p. These genes are deleted in various epithelial cancer cells or tumors at different frequencies. In general, the suppressor activity of the genes has been detected by introducing the given gene into cancer cells, resulting in reduced growth rate in immunosuppressed mice. Numbers refer to publications dealing with the subject and are listed in the references. See text for further details and additional references. K/O: knockout mice.*

Gene	Inactivation by mutation	Inactivation by methylation	Haplo-insufficiency	Suppressor activity	Tumors in K/O
VHL	35	36			37
RARB		38		39	
TGFBR2	11	40		41	
MLH1	42	9			43
HYA22	44			44	
CTNNB1	45				
LIMD1				33	
LTF		42		32	
SEMA3B		47		48	
HYAL1		49		49	
RASSF1		50		51	
BAP1				52	
FHIT		18	20	19	20
ROBO1		46		53	46

of the fragile site in the breast tumor cell, but could also be in line with the tumor suppressor role of FHIT. It may be questioned whether the fragile sites in the genome are more sensitive to alterations in a BRCA1 and BRCA2 mutation background due to the DNA repair role of Brca1 and Brca2. This could be part of the story, but not the only explanation. When comparing the deletion data from chromosomes that carry the most common fragile sites in the genome, FRA3B, FRA16D and FRA6E, only chromosomes 3p and 6q show elevated LOH in BRCA2-associated tumors compared to sporadic breast tumors, but not chromosome 16q (26). Also, there is higher frequency of deletions at chromosome 8p in BRCA2-associated tumors than in sporadic tumors, even though this chromosome region does not contain a defined fragile site (27). The architecture of 3p deletions is different in sporadic as opposed to BRCA2 tumors (21).

Deleted regions at 3p in human tumors and the elimination test

In addition to loss of certain TSGs like VHL and FHIT in human tumors, various deleted regions at 3p have been described, such as AP20, LUCA and U2020 (Figure 1). These regions carry putative TSG or TSGs. Imreh *et al.* (28) also developed an assay based on the non-random elimination of human chromosome segments in mouse-human microcell hybrids during tumor growth in SCID mice. This assay, called the elimination test (Et), was designed for the identification and fine-mapping of chromosomal regions containing putative TSGs. Microcell hybrids of either mouse fibrosarcoma or human renal cell carcinoma cell lines, with an intact or partially deleted human chromosome 3, were generated (28, 29). After *in vitro* cultivation and *in vivo* passage in SCID mice, the tumors were examined and retained human chromosome 3 sequences were identified. By using the Et, it was possible to identify commonly lost segments, termed CER2 at 3p22, CER1 at 3p21.3 and FER at 3p21.1-p21.2 (30) (Figure 1). The functional role of the genes located at these regions is poorly-defined in relation to tumor pathogenesis.

The LTF gene within the CER1 is a potential TSG, as is the LIMD1. LTF can suppress the growth of a fibrosarcoma cell line and of v-ras-transformed NIH3T3 cells, and it inhibited experimental metastasis of melanoma cells in mice (31). In tumors derived from a fibrosarcoma cell line previously transfected with LTF, the RNA expression of it was decreased or eclipsed. It was asserted that promoter methylation and/or chromosome rearrangements at the insertion site were responsible for this LTF down-regulation (32). The LIMD1 gene belongs to a group of LIM domain-containing protein families, for which a function has been postulated in

intracellular signaling pathways, transcriptional regulation and cellular differentiation. The Limd1 binds the tumor suppressor pRb and represses E2F-driven transcription (33). LIMD1 blocks tumor growth *in vitro* and *in vivo* and is down-regulated in the majority of lung cancer samples analyzed (33).

Although the involvement of the cluster of eight chemokine receptor genes (CCR) at CER1 in tumor initiation and/or progression is unclear, it is possible that the elimination of CCRs facilitates a tumor's escape from infiltrating phagocytic macrophages or from neutrophils that secrete CCR-binding ligands. The findings of Manes *et al.* (34) showed that CCR5 is a regulator of TP53 transcriptional activity in breast cancer cells. Blocking of CCR5 enhanced the proliferation of xenografts from tumor cells with wild-type TP53.

About 3 Mb centromeric to CER1 is the LUCA region, which contains several genes of interest, for example RASSF1, HYAL1 and SEMA3B (Figure 1). Telomeric to CER1 are regions that have been detected with homozygous deletions in human malignancies.

We have addressed the question of whether the CER1 region is preferentially lost in human tumors, as was evident using the Et model system (29). We also sought to determine whether the fragile nature of FRA3B induces terminal deletions leading to 3p14.2–pter losses, or whether the eventual 3p21.3 losses are interstitial. We analyzed and compared the frequency of deletion of the CER1 region with that in two other tumor suppressor regions on 3p: the 3p14.2 region, which contains the FHIT, and the VHL region, which maps near the telomere to 3p25.3. Our results show that chromosomal deletions at CER1 are a very common event in multiple human tumors, and that interstitial deletions prevail (5). Therefore, the chromosome regions defined by the Et are also frequently deleted in human tumors (5). Certain additional regions at 3p are frequently deleted in human tumors, such as the previously mentioned LUCA region, but are not preferably eliminated in the mouse model. Perhaps this reflects a more complicated pathogenesis in the human cancer, for example cell-cell or cell-matrix interactions. Indeed, several genes at LUCA participate in cell-matrix interaction processes, such as the HYAL and SEMA3 genes (Figure 1).

Recent developments in defining tumor suppressor activity from 3p.

The number of putatative TSGs at 3p has been rapidly increasing in recent years, and more than a dozen have been suggested (Table I). Only a few of these genes are clearly mutated in tumor pathogenesis, yet most of them are deactivated by an epigenetic mechanism (Table I). An additional gene at 3p, with putative TSG activity and silenced by both epigenetic and genetic mechanisms, is BLU (54). The well-characterized TSGs VHL and MLH1 are inactivated by a double-hit mechanism, *i.e.* both copies of the given gene are deactivated during tumor progression (35, 55). FHIT is an excellent example of haplo-insufficiency in tumor pathogenesis, as has been well defined in knockout mice (20). Corroborating the role in tumor suppression, many of the genes at 3p show tumor suppressor activity when introduced to cancer cell lines that normally grow as tumors in immunosuppressed mice, but fail to do so when expressing the given gene (Table I). Another indication of TSG activity comes from knockout mice experiments that have been developed for the VHL, MLH1, FHIT and ROBO1 genes (Table I). In general these mice develop tumors.

Conclusion

At the chromosome arm 3p there seems to be a distribution of several TSGs, where some are located in clusters. Few of them show all the major characteristics of a TSG, while deactivation of others has probably only a weak effect on the cancer phenotype. Therefore, some of the TSGs at chromosome arm 3p could be classified as cancer modifiers. Possibly a common loss of more than one of the genes at 3p results in tumor progression, *i.e.* synergism of TSG losses is a key event. Studies on TSGs at chromosome arm 3p gives an excellent opportunity to gain a better understanding of such synergism. However, this is contingent upon establishing the role of the single gene products. Hanahan and Weinberg (56) postulated several biological characteristics of tumor growth, including self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis and evading apoptosis. In relation to synergistic TSG losses, it is of interest that several of the TSGs at 3p are involved in related cellular processes, such as TGFBRII and RASSF1 in anti-growth control, HYAL genes in cell-matrix interactions, VHL and SEMA3 in angiogenesis and FHIT in apoptosis and cell cycle arrest. Information on inactivation mechanisms of TSGs at 3p, in addition to loss of chromosome regions, has been increasing in recent years, *i.e.* mutations and methylations. However, the search is ongoing for the deactivation mechanism in novel TSGs. In addition, only a few of the TSGs at 3p have been analyzed in knockout mice, and it will be of interest to include new putative TSGs in these animal models. Tumor progression and clinico-pathology are clearly associated with 3p alterations and the clinical importance of this genetic information is emerging.

Therefore, molecular pathways should be studied further. Improved understanding of the gene function in relation to tumor cell turnover, cell-cell interaction, cell-matrix interaction and therapy resistance will be important for effective cancer management. Molecular and genetic data have become useful in cancer treatment and this field is already touching the edge of pharmacogenomics. With

increasing knowledge of gene activities and genomics of cancer cells, further development in this direction should be expected in the near future.

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