Interstitial Deletions Including Chromosome 3 Common Eliminated Region 1 (C3CER1) Prevail in Human Solid Tumors from 10 Different Tissues

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A human chromosomal segment regularly lost during tumor formation of microcell hybrids in SCID mice has been mapped to 3p21.3. This segment, called chromosome 3 common eliminated region 1 (C3CER1, also referred to as CER1), may harbor multiple tumor-suppressor genes. Because it was found that similar regions were eliminated in an inter- and intraspecies system and in two tumor types (mouse fibrosarcoma and human renal cell carcinoma), we hypothesized that the importance of C3CER1 would transgress tissue specificity, that is, it could occur in tumors derived from multiple tissues. To evaluate the loss of C3CER1 in various human tumor types, we conducted loss of heterozygosity (LOH) analysis of 576 human solid tumors from 10 different tissues and compared the frequency of deletion in the C3CER1 area to that in two other regions on 3p: the *FHIT*/*FRA3B* region, at 3p14.2, and the *VHL* region, at 3p25.3. Deletions were detected in the C3CER1 region in 83% of informative tumors. Half (47%) the LOH-positive tumors showed LOH at all informative markers, indicating a large deletion. The other half (53%) had a discontinuous LOH pattern, suggesting interstitial deletions or breakpoints. The proportion of tumors with C3CER1 deletions was high in all tumor types investigated, ranging from 70% to 94%, except for the soft-tissue sarcomas (40%). In the *VHL* and *FHIT* regions, deletions were observed in 73% and 43%, respectively, of the tumors. Of the three 3p regions analyzed, the highest deletion frequency was observed in the C3CER1 region. Furthermore, we demonstrated that the interstitial deletions including C3CER1 prevail over 3p14.2–pter losses in solid tumors.

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INTRODUCTION

Chromosome 3 is abnormal in a variety of human cancers, and several candidate tumor-suppressor genes (TSGs) on 3p have been described (Braga et al., 2003; Imreh et al., 2003). Hemi- and homozygous deletions in various tumorigenic cell lines and carcinoma tissues provide further support for the presence of TSGs on 3p (Kovacs et al., 1988; Kok et al., 1994; Eiriksdottir et al., 1995; Lifu et al., 1996; Petursdottir et al., 2002).

Imreh et al. (1994) developed an assay based on nonrandom 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 A9 mouse fibrosarcoma or human renal cell carcinoma (RCC) cell lines with an intact or partially deleted human chromosome 3 have been generated (Imreh, et al., 1994). After in vitro culti-

vation 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 a commonly lost segment, termed chromosome 3 common eliminated region 1, or C3CER1 (referred to also as CER1; Kholodnyuk et al., 1997). A detailed transcriptional map of this region, which spans ~1.4 Mb at 3p21.3, has been published (Szeles et al., 1997; Yang et al., 1999; Kiss et al., 2002). According to this map, the ~1.4-Mb C3CER1 region covers 19 active genes

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and 3 pseudogenes. The genes, as listed by the transcriptional map, are: *KIAA0028*, *LIMD1*, *KIAA0851*, *XT3*, *LZTFL1*, *FYCO1*, *LTF*, *TMEM7*, *LRRC2*, *LUZP3*, *TDGF1*, and a cluster of 8 chemokine receptors (*CCRs*) (Kiss et al., 2002). The functional role of these genes in tumor formation is still unknown.

The lactoferrin (*LF*) gene is a potential TSG, as is the LIM domain containing 1 gene (*LIMD1*; Imreh et al., 2003). *LF* 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 (Bezault et al., 1994). In tumors derived from a fibrosarcoma A9 cell line previously transfected with either *LF* cDNA or the full-length *LF* gene, *LF* RNA expression was decreased or eclipsed. It was asserted that promoter methylation and/or chromosome rearrangements at the insertion site were responsible for this *LF* down regulation (Yang et al., 2003). The novel *LIMD1* gene identified by Kiss et al. (1999) belongs to a group of LIM domain-containing protein families that have been proposed to function in intracellular signaling pathways, transcriptional regulation, and cellular differentiation during development (Crawford et al., 1994). A tumor cell line panel was Northern blot-hybridized with *LIMD1*. Several truncated transcripts were detected in most of the tumor cell lines. In some cases, all 3 LIM domains were missing (Kiss et al., unpublished), suggesting its tumor-suppressor function.

Although the involvement of the CCR cluster (*CCR9*, *STRL33*, *CCXCR1*, *CCR1*, *CCR3*, *CCR2*, *CCR5*, and *CCRL2*) in tumor initiation and/or progression is unclear, it is possible that the elimination of CCRs could facilitate a tumor's escape from infiltrating phagocytic macrophages or from neutrophils that secrete CCR-binding ligands. Recent findings of Manes et al. (2003) showed that *CCR5* is a regulator of *TP53* transcriptional activity in breast cancer cells. Blocking of *CCR5* enhanced proliferation of xenografts from tumor cells with wild-type *TP53*.

About 3 Mb centromeric to C3CER1 is the LUCA region, which contains several genes of interest, for example, *RASSF1*, *HYAL1*, and *SEMA3B*. Telomeric to C3CER1 are regions that have been detected with homozygous deletions in human malignancies (Imreh et al., 2003). Other 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. Inactivation

of the *VHL* gene is a prevalent genetic alteration in both hereditary and sporadic renal carcinomas. Frequent LOH and terminal 3p deletion including the *VHL* region have been reported in multiple malignancies, including renal cell, ovarian, and endometrial carcinomas (Maynard and Ohh, 2004).

The *FHIT* gene, at 3p14.2, contains a common fragile site, FRA3B. We previously investigated LOH frequency at the *FHIT* locus, using the same tumor material as used in the current investigation. In the previous study, LOH at the *FHIT* gene affecting at least one of the six analyzed markers was detected in half the informative tumors, ranging from 30% to 66%, depending on tumor location (Ingvarsson et al., 2001; Petursdottir et al., 2002). LOH at all the informative *FHIT* markers was more prevalent than breakpoints, suggesting a large deletion. Thus, these data indicated that LOH at the *FHIT* gene is a fairly common event in all the tumor types analyzed. However, the question remained whether the LOH resulted from mitotic nondisjunction, mitotic recombination between the homologues or nonhomologues, or gene conversions, or whether the LOH disclosed terminal or interstitial deletions. The presence of the FRA3B may suggest that terminal deletions occur frequently and may lead to 3p14.2-pter losses. The pericentromeric 3p10-p11 region has preferential breakpoints in many solid tumors that may lead to entire 3p losses, as has been proven in model systems as well as by analysis of databases (Kost-Alimova et al., 2004).

In this investigation, we addressed the question whether the C3CER1 region at 3p21.3 is preferentially lost in human tumors, as was evident using the Et model system (Imreh et al., 1994). 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 C3CER1 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. Because the tumor material used in the current investigation of LOH in the 3p25.3 and 3p21.3 regions was the same as that used in our previous study of LOH at the *FHIT* locus (Ingvarsson et al., 2001; Petursdottir et al., 2002), we used the previous data for comparison of LOH frequency. Our results suggest that chromosomal deletions at C3CER1 are a highly common event in multiple human tumors and that interstitial deletions prevail.

MATERIALS AND METHODS

Patients and Tumor Material

Samples from 576 primary solid tumors and matching nonmalignant tissue were obtained on the day of surgery. The samples were immediately frozen at -70°C . The organs from which the tumors came and the number of each were: breast (159), stomach (38), colon/rectum (115), lung (70), endometrium (25), ovaries (42), testicles (31), kidney (67), thyroid gland (14), and soft-tissue sarcomas (15). All information on the tumors was recorded at the Department of Pathology, Landspítali University Hospital, Iceland.

DNA Extraction and PCR

Tumor and normal DNA was extracted from the tissue samples with proteinase K using a method developed for paraffin-embedded tissue (Smith et al., 1992). Paired samples of tumor and normal DNA were subjected to PCR analysis. DynaZymeTM polymerase (from Finnzymes Oy, Espoo, Finland) was used in the buffer solution provided by the manufacturer. Samples were denatured for 3 min at 95°C and then subjected to 35 cycles of amplification consisting of 30 sec at 95°C , 30 sec at 55°C – 60°C , and 60 sec at 72°C , followed by 10 min at 72°C .

The microsatellite markers in the C3CER1 region that were used were *D3S3582*, *D3S2354*, *AFMB362WB9*, *GAAT-p33715*, and *D3S1767*. *D3S3582* is centromeric to the *RIS1* gene, near *LARS2*; *D3S2354* is in a terminal intron of *LARS2*; *AFMB362WB9* is between two CCRs, *CCXCR1* and *CCR1*; *GAAT-p33715* is between *LF* and *TMEM7*; and *D3S1767*, already outside CER1, is near the *MGC2391B* gene. Therefore, of the five markers, four are within C3CER1, and one (*D3S1767*) is about 0.3 Mb outside the region toward the centromere. The C3CER1 region covered by the markers is a total of 1.5 Mb.

As a control, we used three markers (*D3S4545*, *D3S1317*, and *D3S1335*) that map to 3p25.3; these markers cover 1.7 Mb of DNA around the *VHL* region (Fig. 1). Also as a control, we used the published results on four microsatellite markers within the *FHIT* gene, covering 0.67 Mb (Ingvarsson et al., 2001; Petursdottir et al., 2002). According to the Ensembl genome browser at the Sanger Center, the markers in the *VHL* region that were used are external to the *VHL* gene and cover at least 29 genes. The C3CER1 region markers used cover 19 active genes and 3 pseudogenes, and the *FHIT* markers are all within the *FHIT* gene.

LOH Analysis

The PCR products were separated on 6.5% polyacrylamide 8 M urea gels in $1\times$ TBE buffer for 3–4 hr at 60 W. After electrophoresis, the PCR products were transferred to a Hybond-N⁺ nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). Primers were elongated by terminal-transferase in 40 mM K-HEPES/1 mM CoCl_2 buffer at pH 7.2 and 37°C overnight to facilitate the binding of peroxidase to the probe (Vignal et al., 1993). The Hybond-N⁺ membranes were then hybridized for at least 2 hr at 42°C with elongated primers and covalently labeled with peroxidase (ECL labeling kit, Amersham Pharmacia Biotech). After hybridization, the membranes were washed once for 20 min in $3\times$ SSC/0.1% SDS at 42°C and then twice for 15 min in $0.2\times$ SSC at 42°C . Finally, the membranes were bathed in a detection reagent containing H_2O_2 , luminol, and an enhancer (ECL detection kit, Amersham Pharmacia Biotech) for 1 min at room temperature, and signals were detected on AGFA Cronex-5 film. Films were inspected visually; any absence or significant decrease in the intensity of one allele relative to the other was considered LOH.

RESULTS

LOH Analysis of the 3p21.3 (C3CER1) Region

The microsatellite polymorphism at the C3CER1 locus was analyzed by screening 576 human solid tumors using five C3CER1 microsatellite markers (Fig. 1). The majority (408) of analyzed samples were informative for the C3CER1 markers. As presented in Figure 2, we detected LOH for at least one C3CER1 marker in 339 (83%) of the 408 informative tumors. All informative C3CER1 markers were deleted in 158 (47%) of the LOH-positive tumors, indicating a large deletion. The remaining 181 tumors with LOH had a discontinuous LOH pattern, suggesting interstitial deletions, or breakpoints, in the C3CER1 region. The LOH frequency for at least one marker was high, ranging from 70% to 94% LOH, in tumors from all locations except for the soft-tissue sarcomas, the proportion of which that had LOH was only 40% (Fig. 2). It should be noted, however, that the sample size analyzed for endometrial and thyroid tumors as well as soft-tissue sarcomas was rather small, explaining less significant results (Table 1). There was a difference in the observed LOH frequency for individual C3CER1 markers depending on tumor location. The highest was 80% at the *GAAT-p33715* marker in testis tumors, and the lowest was

Figure 1. Schematic representation of 3p with the locations of markers used in this study. The C3CER1 region at 3p21.3 and the chromosomal area it spans are shown in megabases from the telomere, as is information on marker location. The number of informative samples and the proportion of tumors with LOH are given for each marker.

0%, which occurred at the same marker in endometrial tumors and at the *AFMB362WB9* marker in thyroid tumors (Fig. 3).

LOH Analysis of the 3p25.3 (VHL) Region

As a control and to assess the dimension of the critical region as well as to determine if our results suggested terminal or interstitial deletions, we compared LOH frequency in the C3CER1 region to that in two other regions on 3p. One region was 3p25.3, where the *VHL* tumor-suppressor gene resides. We used three microsatellite markers mapped around the *VHL* gene (Fig. 1). Only 320 (of 576) tumors were informative (Table 1), and of these, 232 (73%) had deletions at the site of at least one *VHL* marker (Fig. 4). Of the 232 LOH tumors, 126 had LOH at all informative *VHL* markers.

Therefore, 106 (54%) of the LOH tumors had a discontinuous LOH pattern. The deletion frequency in the *VHL* region was high in tumors from all analyzed organs, ranging from 63% in stomach tumors to 93% in kidney tumors (Fig. 4).

Comparison of LOH Frequency at C3CER1 to VHL and FHIT

The total LOH frequency (of all tumor types pooled together) in the C3CER1 region was significantly higher than the LOH frequency in both the *FHIT* (data from our previous studies: Ingvarsson et al., 2001; Petursdottir et al., 2002) and the *VHL* regions ($P < 0.05$ in both cases; Fig. 5). Similarly, the total LOH frequency at the *FHIT* locus was significantly lower than that at the *VHL* locus ($P < 0.05$). If the tumors were separated according to organ type,

■ C3CER1 LOH at one or more markers □ C3CER1 LOH at all informative markers

Figure 2. Graphic presentation of C3CER1 LOH frequency in tumors from 10 different tissues. Shown are the percentages of samples with LOH of at least one (black bar) or all of the informative markers (gray bars).

LOH frequency in the C3CER1 region was significantly higher ($P < 0.05$) than that at the *FHIT* locus in all tumor types except in tumors with a low sample number, that is, endometrial and thyroid tumors and soft-tissue sarcoma. The deletion frequency was higher in the C3CER1 region than at the *VHL* locus in all tumor types analyzed except for endometrial, ovarian, and renal tumors and soft-tissue sarcoma (Fig. 5). However, this difference was significant ($P < 0.05$) in only three tumor types: breast, gastric, and lung. The deletion frequency at the *VHL* locus in all the investigated tumor types was higher than that observed for *FHIT* and was statistically significant in breast, colorectal, ovary, and renal tumors and in sarcomas (Fig. 5).

Breakpoint Analysis of the C3CER1 and *VHL* Regions

In tumors with a discontinuous LOH pattern, breakpoints can be located within the region analyzed. Thus, with the exception of the thyroid tumors and the soft-tissue sarcomas, we were able to locate breakpoints in the C3CER1 region in 44–64% of the samples that had LOH (Table 2). Our results suggest hot-spot regions for breakage between markers *D3S3582* and *D3S2354* and between *GAAT-p33715* and *D3S1767*, that is, toward both ends of the C3CER1 region (Fig. 6a).

Breakpoints in the *VHL* region were located in 19–67% of tumors with LOH (Table 3), again excluding thyroid tumors and soft-tissue sarcomas. *D3S1335* and *D3S1317* are both close to the *VHL* gene, 0.87 and 0.41 Mb downstream, respectively. *D3S4545* is much farther from the *VHL* gene, approximately 1.6 Mb upstream. Even though the area covered by *D3S1335* and *D3S1317* is significantly smaller, we found nearly twice as many breakpoints as were found between *D3S1317* and *D3S4545*: 56 versus 32 breakpoints, respectively (Fig. 6b).

To determine the prevalence of loss of the whole 3p or of 3p14.2–pter deletions, we analyzed the proportion of tumors that showed loss of all informative markers typed in the three investigated regions. Of the 417 samples analyzed (not including breast samples), 208 were informative for all the markers in the three regions analyzed. Of these tumor samples, only 27, or 13%, had lost all informative markers at all the 3p loci investigated (results not shown), suggesting that loss of the whole 3p arm or 3p14.2–pter deletions is relatively rare and that the 3p losses are predominantly interstitial deletions.

DISCUSSION

The Et is an assay designed for the identification and fine-mapping of chromosomal regions containing putative TSGs (Imreh et al., 1994). Using this

Figure 3. Comparison of LOH frequency for all *VHL* and C3CER1 microsatellite markers in tumors originating from 10 different tissues. Microsatellite markers around the *VHL* region are shown in black and gray; markers at C3CER1 are shown in color.

TABLE I. Total Number of Tumors for Each Tissue and Number of Tumors Informative for C3CER1 and *VHL* Markers

Tumor location	Number of tumors	C3CER1 informative tumors (%)	<i>VHL</i> informative tumors (%)
Breast	159	127 (80)	114 (72)
Gastric	38	26 (68)	16 (42)
Colorectal	115	76 (66)	53 (46)
Lung	70	52 (74)	39 (56)
Endometrium	25	20 (80)	12 (48)
Ovary	42	23 (55)	22 (52)
Testis	31	19 (61)	16 (52)
Renal	67	45 (67)	28 (42)
Thyroid	14	10 (71)	10 (71)
Soft-tissue sarcoma	15	10 (67)	10 (67)
Total	576	408 (71)	320 (56)

assay, a regularly deleted region of 3p21.3, C3CER1, was identified. The transcriptional map of C3CER1, containing 19 genes, has been published (Kiss et al.,

2002). The Et was initially performed using human chromosome 3/mouse fibrosarcoma (Imreh et al., 1994, 2003), then continued with human chromosome 3/human RCC material (Yang et al., 2001). In both systems, similar eliminated regions have been detected. This suggests that the importance of genomic losses including that of C3CER1 transcends species and tissue specificity and may occur nonrandomly in tumors derived from multiple tissues. To investigate the validity of this hypothesis, we conducted an LOH analysis, using reliable tumor material of 576 human solid tumors from 10 different tissues. To assess the dimension of the critical region and to determine whether the fragile nature of *FRA3B* at 3p14.2 induces terminal deletions leading to 3p14.2-pter loss, we compared the deletion frequency of this area to two other tumor-suppressor regions on 3p, namely, the *FHIT* region, at 3p14.2, and the *VHL* region, at 3p25.5.

Of the 576 tumors analyzed, 408 were informative for the C3CER1 markers used. A total of 339

Figure 4. Graphic presentation of *VHL* LOH frequency in tumors from 10 different tissues. Shown are the percentages of samples with LOH of at least one (black bar) or all of the informative markers (gray bars).

Figure 5. Comparison of LOH frequency of at least one marker at three 3p regions. The *FHIT* gene is at 3p14.2 (light gray bars), the *C3CER1* region is at 3p21.3 (black bars), and the *VHL* region (dark gray bars) is at 3p25.3.

a) C3CER1 region

b) VHL region

Figure 6. The location and number of identifiable breakpoints. Distance between markers is shown in megabases. (a) C3CER1 region (339 informative samples), in which the breakpoints cluster toward both of this region's borders. (b) VHL region (220 informative samples) with approximate location of the VHL gene shown. Breakpoints are more common downstream of the VHL gene (toward centromere) than upstream of it.

TABLE 2. Percentage of Located Breakpoints in the C3CER1 Region

Tumor locations	Number of LOH samples	Number of LOH samples with breakpoints within C3CER1	Breakpoints between C3CER1 markers (%)	Breakpoints outside C3CER1 markers (%)
Breast	107	57	53	47
Gastric	24	11	46	54
Colorectal	58	36	62	38
Lung	49	22	45	55
Endometrium	14	9	64	36
Ovary	17	10	59	41
Testis	17	10	59	41
Renal	41	18	44	56
Thyroid	8	6	75	25
Soft-tissue sarcoma	4	1	25	75
Total	339	180	53	47

tumors had LOH with at least one marker. Of these, 158 showed LOH with all the informative markers, possibly as a result of a larger deletion. The remaining 181 LOH tumors showed a discontinuous LOH pattern, in which case breakpoints

could be located using the analyzed markers in the C3CER1 region. Only 320 tumors were informative for the three VHL markers used. More than two-thirds had deletions at one or more markers. Approximately half the LOH tumors had lost all

TABLE 3. Percentage of Located Breakpoints Around the VHL Region

Tumor locations	Number of LOH samples	Number of LOH samples with breakpoints around <i>VHL</i>	Breakpoints between <i>VHL</i> markers (%)	Breakpoints outside <i>VHL</i> markers (%)
Breast	79	44	56	44
Gastric	10	3	30	70
Colorectal	36	17	47	53
Lung	28	9	32	68
Endometrium	9	6	67	33
Ovary	18	11	61	39
Testis	12	5	42	58
Renal	26	5	19	81
Thyroid	7	6	86	14
Soft-tissue sarcoma	7	0	0	100
Total	220	90	41	59

informative markers; the other half had a discontinuous LOH pattern. The deletion frequency in the *VHL* region was high in tumors from all of the analyzed organs.

D3S1767 is about 0.3 Mb outside the centromeric border of C3CER1 toward the LUCA region described by Lerman and Minna (2000). The LUCA region is an interesting segment at 3p21.3; it is defined by homozygous deletions in small cell lung carcinoma, non-small cell lung carcinoma, breast, kidney, and other carcinomas and contains 25 genes (Lerman and Minna, 2000), at least 7 of which are candidate TSGs, with *RASSF1* the most suggestive so far (Imreh et al., 2003). The observed LOH at the *D3S1767* marker was high, suggesting that LOH in this tumor material could also be high for the LUCA region, prompting further analysis using this material.

Comparing LOH frequency in the C3CER1 region to that at other loci was an important control. This allowed us to deduce whether the LOH frequency was in fact elevated in this particular area. One locus that we used as a control is *FHIT*, a TSG at 3p14.2. An aphidicolin-sensitive common fragile site (FRA3B) maps within the *FHIT* gene, which makes it susceptible to chromosomal breakage and rearrangements; therefore, *FHIT* can be linked to hyperinstability. Although a number of studies have demonstrated *FHIT* to have a tumor-suppressor function (Siprashvili et al., 1997; Ji et al., 1999; Sard et al., 1999; Werner et al., 2000; Dumon et al., 2001; Ishii et al., 2001; Zanesi et al., 2001), the relatively high LOH frequency that we observed in our previous studies (Ingvarsson et al., 2001; Petursdottir et al., 2002) could be, at least in part, a result of the naturally occurring fragility of the area. Given that the *FHIT* locus at 3p14.2 is classified as fragile (see below) and that the LOH frequency at

C3CER1 is significantly higher than at the *FHIT* gene strongly suggest that LOH at C3CER1 is a causative element in tumor development rather than a reflection of an unstable genome in tumor cells. Furthermore, our results suggest that only 13% of any 3p14.2-pter loss in our tumor material could possibly result from breakage in the *FHIT*/FRA3B region (all informative markers at the 3 regions analyzed lost).

According to Richards (2001), the C3CER1, at 3p21.3, is not a genomic region classified as a fragile site, that is, it does not meet the criterion of being fragile under conditions that cause late replication. However, the centromeric segment of the C3CER1 has been shown to contain a murine/human conservation breakpoint region (CBR), which means that the equivalent region in the mouse is split into two segments on mouse chromosome 9. The CBR region has instability features because it is regularly deleted in YACs, it has gene duplications and pseudogenes, and it has an elevated frequency of long terminal repeat insertions and a hairpin-forming TATAGA repeat that may be associated with deletions. It also was observed, using a FISH-based replication-timing analysis, that DNA replication of the CBR region is delayed (Kost-Alimova et al., 2003). Assuming that both the *FHIT* and the C3CER1 regions have features that predispose to fragility, although on a different background, we can conclude that loss of the C3CER1 imparts additional selective growth advantage.

Breakpoint is a term used to identify juxtaposed regions in which one region has LOH and the other has ROH (retention of heterozygosity). We found that approximately half the tumors with LOH in the C3CER1 and *VHL* regions had a discontinuous LOH pattern. One third of tumors with discontin-

uous deletions in the C3CER1 region had retained one or more markers around the *VHL* gene. There appear to be hot-spot regions for breakage toward both ends of the C3CER1 region. This could reflect a synergism of TSG loss in the C3CER1 region with tumor pathogenesis.

The *D3S1335* and *D3S1317* markers are both close to the *VHL* gene, 0.87 and 0.41 Mb downstream, respectively. *D3S4545* is approximately 1.6 Mb upstream from the gene. Therefore, if deletions were random in this region, we would expect breakpoints to be more common between *D3S1317* and *D3S4545* (which are more than 1.6 Mb apart) than between *D3S1317* and *D3S1335* (which are only 0.46 Mb apart). However, we found the opposite to be true. Almost twice as many breakpoints were found between markers *D3S1335* and *D3S1317* as found between *D3S1317* and *D3S4545* (Fig. 6), implying that breakage occurring near the *VHL* gene confers a greater growth advantage for tumors than does breakage telomeric to the gene. It would be of interest to analyze the DNA sequence in these breakpoint regions in order to define elements responsible for the breakage.

Several TSGs seem to be located at 3p21.3 that could be targets in some cancers but not in others. The high number of genes in the C3CER1 and *VHL* regions possibly could explain in part why the LOH is so high there compared to that in the *FHIT* region.

Taken together, our data indicate that LOH in the C3CER1 region was more frequent than was LOH in either the *FHIT* or the *VHL* regions in the vast majority of the 10 tumor types analyzed. LOH at C3CER1 was widespread in all tumor types investigated, indicating that C3CER1 deletions transgress tissue specificity. Still, it cannot be excluded that certain deletions may be characteristic of a specific tumor type. Furthermore, we showed that only 13% of the tumors had lost all informative 3p markers, demonstrating that interstitial deletions including deletions in C3CER1 prevailed.

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