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More Is Less: Inactivation and Deletion Events and the Search for Tumor Suppressor Genes

W. Edward C. Bradley,[1*] Domenic Di Paola,[2] Emmanouil Rampakakis,[2] and Maria Zannis-Hadjopoulos[2]

[1] *Centre de Recherche du CHUM, Research, JA deSeve Bldg., Montreal, Quebec, Canada H2L 2W5*

[2] *Goodman Cancer Centre & Biochemistry, McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada H3G 1Y6*

ABSTRACT

Tumor suppressor genes are frequently inactivated in cancer by large-scale deletion events or epigenetic silencing, and experimental demonstration of such inactivation has historically been considered as support for assigning tumor suppressive function to a given gene. However, the discovery of a number of chromosomal domains wherein large deletions naturally occur at frequencies up to 100 times the average for the genome as a whole leads us to reevaluate the significance of sporadic deletions found within genes associated with these hotspots. Similarly, our recent demonstration that epigenetic chromatin silencing frequently spreads in cancer cells from gene-poor into gene-rich regions with apparent indifference to the gene content of the affected domain raises questions about the pertinence of inactivation as a criterion for ascribing tumor suppressor function to a given gene. We suggest that a number of putative suppressor genes for which inactivation and/or deletion events have been documented may simply be victims of collateral damage when these events occur, and the implication that these genes are being selected against during cancer progression should in some cases be reassessed. J. Cell. Biochem. 110: 281–287, 2010.

KEY WORDS: DELETIONS; EPIGENETIC SILENCING; TUMOR SUPPRESSOR GENES; HIGH FREQUENCY; GENOME-WIDE; CHROMATIN IMMUNOPRECIPITATION; TUMOR CELLS VERSUS NORMAL CELLS

Events reflecting genomic instability, including large deletions and epigenetic silencing, play crucial roles in the initiation and progression of cancer through functional inactivation of tumor suppressor genes (TSGs). The logical extension of this, namely the concept that genes which are frequently inactivated or deleted in cancer probably have suppressive effects on tumor development, has become a fundamental tool in the ongoing search for TSGs. This led to many notable advances beginning with the cloning of the prototypical TSG, RB1, by exploiting large cytogenetically detectable deletions in chr13q14 found in individuals with retinoblastoma. Altered transcriptional activity has also led to identification of candidate TSGs, as reduced expression frequently coinciding with methylation of promoter-associated CpG islands (CGIs) has been documented for many genes in cancer. The dogma which has emerged is that inactivation/deletion equals candidate TSG status. But the mere existence of such a change in a tumor is of course insufficient to support far-reaching conclusions regarding a central role in cancer for a given gene. It is almost certain that, just as for point mutation, so-called drivers and passengers exist within these categories of tumor-associated changes, the former being those changes contributing significantly to the cancer phenotype, the latter being chance occurrences with no important role to play.

A variety of criteria exist to assess whether a given mutation or inactivating event may be a driver, but that which is usually the first to be satisfied is its presence in a relatively high proportion of those cancers or cancer-derived cell lines which were examined. Once a significant frequency of inactivation has been established, the effort required for functional studies can be justified.

In this Prospects review, we wish to address two aspects of long-range chromosomal instability and explore how recent conceptual advances in our understanding should influence current and future conclusions with respect to assigning candidate tumor suppressor status to a given gene.

*Correspondence to: W. Edward C. Bradley, Centre de Recherche du CHUM, Research, JA deSeve Bldg., 1560 Sherbrooke E., Montreal, Quebec, Canada H2L 4M1. E-mail: edward.bradley@umontreal.ca

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STRUCTURAL ALTERATIONS AND HOTSPOTS OF DELETION

The search for candidate TSGs through the mapping of large-scale deletions (kb to Mb range) has received a boost in recent years through development of technologies enabling unbiased genome-wide assessment of these and related events (collectively referred to as copy number variations, CNVs). Currently available microarrays from a variety of commercial suppliers allow genome-wide analyses to be performed within the budgets of many academic laboratories. We therefore have the tools to explore at a profound level the molecular lesions responsible for cancer initiation and progression but the plethora of data generated by these technologies presents us with new challenges in differentiating between driver and passenger alterations.

Recent advances in understanding the nature of genetic and epigenetic changes on a genome-wide scale have pointed to an unexpected degree of non-uniformity in the distribution of these events. The initial mapping of CNVs by several groups using high-throughput array technologies, as curated by databases such as the DGV and the UCSC Genome Browser, reveals striking clusters of CNVs at a relatively limited number of sites. The unknown parameter as of now stems from the uncertainty of CNV boundary calling; thus, a deletion occurring frequently in a population may be called as several slightly different events, and the database curators have in the past handled this uncertainty by assigning clusters of very similar CNVs to a locus. However, the available high-resolution data from a variety of sources now point to the existence of "hotspots" of rearrangement where deletions (and to a lesser extent, duplications) occur at frequencies 10- to 100-fold higher than in the neighboring chromosomal domains.

First, in the very long gene DMD, one of the most intensely studied with respect to CNV occurrence, two hotspots have been documented since the mid-1990s. The major hotspot (affecting 0.7 Mb encompassing exons 40–54) undergoes de novo deletions in germ cells at frequencies at least 10 times higher than elsewhere in the 2.4 Mb gene [reviewed in Sironi et al., 2006]. That these are independent events has been exhaustively documented by genetic and molecular approaches.

Second, neurexin 1 (NRXN1), another very long gene, has been implicated in schizophrenia through a highly significant association of CNVs affecting coding sequences in patients. The distribution of these CNVs, again mostly deletions, is striking, with 90% falling in the 5′ half of the 1.1 Mb gene. Although one possible explanation for the skewed distribution is greater biological relevance of the sequences at the 5′ end of the gene, the fact that deletions not affecting any exons at all were similarly distributed (both in patients and controls) suggests that a relatively high state of chromosomal instability was the more likely reason for the clustering.

Third, another very long disease-associated gene, PARKIN, is characterized by clustering of CNVs in Parkinson's patients in a domain of about 0.5 Mb affecting principally exons 3 and/or 4 in 50% of cases, with less frequent loss of exons 2 and 5, followed by progressively lower frequency extending away from this hotspot. A recent publication describes the sequencing of the end-points of 18 deletions found in the exons 2–5 region, and all were different and did not involve such structures as segmental duplications [Asakawa et al., 2009], reinforcing the "hotspot" nature of the chromosomal domain.

Finally, two genome-wide studies of nearly 1,000 individuals each report clustering of deletions in 10–20 hotspots wherein the deletion frequency has been calculated as up to 100-fold higher than the genome as a whole. The report from our laboratory [Bradley et al., 2010] was specifically designed to search for evidence of clustering of rare independent deletions in autosomes, using 440 parent–parent–child trios to allow verification of boundaries of transmitted deletions in both parent and child. This was coupled with human inspection of each image depicting the distribution of fluorescence intensities which was identified as a probable deletion by the software used (PennCNV). This combination yielded a specificity of 98% and a sensitivity of 94% in calling deletions. Deletion boundaries for parent and child were assessed separately and were in perfect agreement for about 99% of the transmitted deletions. This work identified 13 hotspots in the human genome, including those in the NRXN1 and PARKIN genes (above), where deletions, mostly 20–200 kb in length, occur at an average of 50-fold higher frequency than elsewhere. A total of seven de novo deletions were found, five of which were in the hotspots, giving an enrichment over non-hotspot regions of about 1,000-fold. The hotspots were all about 0.5 Mb in length, and their existence was subsequently confirmed in three other population samples for a total of 2,540 unrelated individuals. Poisson distribution analysis projected that up to 30 or more hotspots exist in the genome.

The earlier study by Blauw et al. [2008] did not specifically conclude that hotspots exist, but did present results in sufficient detail that it was possible to perform data-handling similar to that performed in our study. The concordance was remarkable (Table I), with clustering to a similar degree in nearly all of the hotspots we subsequently identified.

IMPLICATIONS FOR TSG DISCOVERY

The consequences of this discovery from a medical genetics perspective are significant, as are the implications for the ongoing search for TSGs. The latter are discussed in this section.

Several of the hotspots found in both sets of data lie in genes or regions which have been implicated in cancer (Table I). A remarkable example is an array CGH study of deletions discovered in colorectal cancers and lines derived from colorectal cancers [Davison et al., 2005], where, respectively, 23% and 55% of the samples had suffered deletions extending over a 2-Mb stretch in 20p12, with the 300 kb consensus overlap region coinciding perfectly with the most unstable of the hotspots we have identified. The authors of this report noted the presence of two untranslated mRNA genes in the region and showed some sequence variations and a reduction in the level of their transcription in cancers. Their interpretation that the very high incidence of deletion of these genes reflected involvement in tumor suppression, while reasonable in the context of knowledge at the time, may be reevaluated in the

TABLE I. Clustering of Large Rare Deletions Detected in Nominally Cancer-Free Individuals in Two Studies; Data Were Handled as Described in Bradley et al. [2010]

Chr	Location of mid-point (mb)	Number Bradley et al. [2010]	Number Blauw et al. [2008]	Total	Fold increase	Gene
1	186.3	1	3	4	19	None
1	235.3	4	0	4	19	None
2	50.9	3	2	5	24	NRXN1
3	4.2	1	3	4	19	None
4	28.1	0	3	3	15	None
6	95.5	3	5	8	39	None
6	162.7	9	3	12	58	PARK2
7	110.7	1	4	5	24	IMMP2L + 1
8	4.6	5	0	5	24	CSMD1
8	5.7	4	3	7	34	None
8	8.4	1	4	5	24	3 Short
8	15.5	3	5	8	39	TUSC3
9	9.7	0	5	5	24	PTPRD
9	11.8	9	6	15	73	None
9	30.5	3	2	5	24	None
10	67.8	7	7	14	68	CTNNA3
13	83.4	8	2	10	49	SLITRK1
14	26.7	2	3	5	24	None
14	104.2	0	4	4	19	4 Genes, short
16	6.7	4	1	5	24	A2BP1
16	76.8	2	5	7	34	WWOX
20	14.8	11	7	18	87	MACROD2
20	40.7	4	2	6	29	PTPRT
Average					34.6	

knowledge of the inherent instability of this domain. The fact that several of the individuals in our study carried germline hemizygous deletions of these genes without any evidence of cancer further argues against a suppressor role for these genes.

The PARKIN gene in band 6q26, has also been assigned a TSG role based largely on the frequency of deletions in cancers. Germline homozygous deletions in this gene cause familial early-onset Parkinson's disease (EOPD), a condition which has not been associated with cancer even in advanced years. As with the deletions in 20p12, those occurring with highest frequency were mapped exactly at the hotspot we identified, around exons 2 and 3 in a study of non-small cell lung cancer [Picchio et al., 2004], and between exons 3 and 5 in hepatocarcinoma-derived lines [Wang et al., 2004]. In both reports, the authors coupled these observations with expression results and a limited number of experiments on the effect of PARKIN on growth or apoptosis, and concluded that the gene was a TSG. A more plausible interpretation, particularly considering the cancer-free state of EOPD patients with homozygote knockout deletions, is that these are passenger mutations occurring in a particularly unstable domain of the genome.

Several other genes, notably IMMP2L, WWOX, CTNNA3, and TUSC3 are associated with hotspots and were found to have exon-disrupting germline deletions in either our study or Blauw et al. [2008], all of which were in individuals free of declared cancer. Each of these has been proposed as a TSG based on the observed frequency of deletions in cancers, and we contend that their status as candidate TSGs should be revised, since our results indicate that the deletions are probably passengers.

It is noteworthy however, that some of the hotspots were in genes wherein no exons were disrupted by any observed deletion. These may indeed be shown to be TSGs in the future, especially those such as the members of the phosphotyrosine phosphatase family, which code for proteins whose function may be consistent with suppressor activity.

OTHER HOTSPOTS PROBABLY EXIST: THE CASE OF 3p21.3

An important consideration in this work is that whereas we have established the existence of hotspots in the genome, we have not identified all of them. Of particular note, there may well be hotspots which will never be found by analyzing genomes of healthy individuals, since we cannot reject the possibility that hotspot domains exist for which any deletion of the size we are detecting is embryonic lethal. How many there may be is in the realm of speculation, but it is interesting to note that the majority of the ~50% of pregnancies which spontaneously terminate in the first month of gestation are thought to be due to genetic defects including lethal deletions. With respect to the arguments presented here however, the important issue is whether some cancer deletion patterns may be explained by the presence of a hypothetical hotspot of deletion which has escaped our notice. These domains would be expected to be gene-rich (hence a deletion would tend to be embryonic lethal and non-detectable in the germline), characterized by high frequencies of deletion in tumors, not necessarily overlapping, and perhaps without a single candidate TSG which fits the criteria better than any of the others in the region. It may also be expected that any search for causative inactivating point mutations would yield little return, for two reasons: first, deletions would predominate for the simple reason that they occur at a much higher frequency than point mutations and secondly, many of the genes would not be genuine targets of carcinogenic (driver) mutation.

One such region may be in band 3p21.3. Since the inception of cytogenetic techniques, deletions have been described in this band in many tumors and tumor-derived cell lines at frequencies which are among the highest described for any recurring chromosomal anomaly in cancer. High-density array CGH shows that some of the deletions are non-overlapping [Dehan et al., 2007], and point mutations are exceedingly rare or non-existent in any of the genes [Lerman and Minna, 2000, and the Cancer Mutation database, http://www.sanger.ac.uk/genetics/CGP/cosmic/]. This is in the face of the remarkable propensity of cancers to accumulate somatic mutations—an average of 80–100 coding sequence alterations per cancer detected for two tumor types [Wood et al., 2007], and more than 20,000 genome-wide point mutations recently reported in each of two cancer-derived lines [Pleasance et al., 2010a,b].

Conventional thinking holds that one reason for the high frequency of deletion is that the region carries several TSGs, and indeed a thorough evaluation of eight of these genes by Hesson et al. [2007] concludes that functional suppressor activity may be ascribed to several of them. However, there is still no consensus as to which if any of the genes in this band are the critical ones. We contend that if this domain is indeed a hotspot, deletion heroic efforts to track down the important TSG may be unjustified, and investments in clinical trials such as that involving FUS1 gene therapy should be reevaluated.

A similar region is in 5q32-33 where deletions have been described in about 15% of cases of myelodysplastic syndrome. The "critical region" spans about 2 Mb in a gene-rich region and several of the deletions are non-overlapping. Again, this could be a hotspot of deletion (perhaps only for cells of the myelogenous lineage) and if so the lack of data identifying this as a hotspot is understandable given the probable lethality of such deletions in the germline.

PATTERNS OF INACTIVATION IN THE CANCER GENOME: THE SPREADING OF EPIGENETIC SILENCING

The second general chromosomal event contributing to cancer progression which we wish to address is epigenetic inactivation.

The documentation of gene inactivation has proven to be a powerful tool in identification of novel candidate TSGs and its usefulness has been enhanced by the application of technology allowing genome-wide searches for such changes. An early advance was the development of Restriction Landmark Genomic Scanning (RLGS), which allows identification of CGIs which are methylated in tumor tissue in a relatively unbiased fashion. Approximately 2,000 CGIs can be screened for methylation changes and several candidate TSGs have been accordingly identified. More recent technologies have advanced the study of genome-wide CGI methylation and are beginning to yield a full picture of the epigenome in normal and cancerous tissues [reviewed in Suzuki and Bird, 2008]. The issue which we wish to emphasize here has to do with the spreading effect of epigenetic inactivation, as this phenomenon has implications for the interpretation of inactivation data involving candidate TSGs.

The classic situations in which epigenetic inactivation spreads over a long distance are X-inactivation and genomic imprinting. These systems have been studied intensively and many excellent reviews are available on these subjects. Of pertinence for this discussion, the inactivation events are generally known to be directed from a defined site (XIC and ICR, respectively), and although most genes in the affected domain (including most of the chromosome in the case of X-inactivation) are transcriptionally silenced, there are many known cases of individual genes escaping inactivation. An intriguing characteristic of the silencing function on the Xi is that upon translocation onto an autosome the inactivation (observable as heterochromatization) can spread into the normally active domains of the autosome.

Frigola et al. [2006] published an important study showing that gene inactivation in cancer could extend over a long distance, in this case the entire 4 Mb chromosome band 2p14.2. This occurred in colorectal cancer and implicated methylation of several, but surprisingly not all of the CGIs in the domain; the chromatin was, however, associated with histone H3K9 dimethylation, a marker of closed chromatin conformation at every site examined, including the unmethylated CGIs. The event was named long-range epigenetic silencing (LRES).

In a commentary on this article, Smith and Costello [2006] raised the question of whether this long-range effect reflected targeted inactivation of one important TSG and the other genes were simply passengers, or whether all genes in the domain may be important in maintaining normal tissue homeostasis. We will argue that it is quite possible that none of the genes are of critical importance for cancer.

More recently, evidence of coordinated inactivation of linked alleles has been reported in breast cancers [Novak et al., 2008]. These authors showed non-random distribution of differentially methylated CGIs in tumors and interpreted the results as supporting the concept of LRES.

These reports of spreading inactivation were foreshadowed by early work on a Chinese hamster ovary-derived fibroblast cell line, CHO [Bradley, 1983; Grant et al., 1989]. In these studies linked genes were shown to undergo coordinated inactivation upon selection against expression at one or the other locus. This event was monoallelic and could occur at high frequency, 10^2- to 10^5-fold higher than point mutation. Inactivation could be incomplete (especially in those cases where the frequency was very high), but the degree of inactivation was equal at the two loci. Dobrovic et al. [1988] showed that this event, dubbed Type II silencing (to distinguish it from single-locus epigenetic inactivation) was independent of promoter methylation at at least one affected gene, but was nevertheless reversed by azacytidine treatment, suggesting the existence of a controlling CpG-rich element elsewhere. All these characteristics, with the exception of the monoallelic nature of the inactivation, are at least partially recapitulated in the LRES event described by Frigola et al. [2006].

Our approach to the question of the distribution of inactivated domains in cancer has been to perform genome-wide genotyping assays on chromatin immunoprecipitated with antiserum against histone 3 acetylated on lysine 9 (H3Ac) compared with that against histone 3 trimethylated on lysine 9 (H3M). These histone modifications are considered to be markers of open chromatin and pericentric heterochromatin, respectively. Four cell sources were used: a culture of normal cells from small intestine, which is

non-clonal and non-immortal, and three lines derived from colorectal cancers.

We used BeadArray microarray platforms (Illumina), which compare intensity of fluorescence of each allele (A or B) to arrive at a value for B allele frequency (BAF) at up to 1 million SNP loci per experiment; these values are then used to call genotypes as AA, AB, or BB. When the hybridized DNA is prepared from chromatin enriched for closed versus open conformation we obtain a measure of both the chromatin conformation at each SNP and the degree to which this conformation differs between alleles. We calculated 21-SNP moving window averages of fluorescence intensities (expressed as Log R values) and plotted these against chromosomal position, yielding patterns of open versus closed chromatin on a megabase scale. We interpret low anti-H3Ac ChIP values coupled with high anti-H3M ChIP as indicating "closed" chromatin.

For the SNPs which were heterozygous in genomic DNA we calculated BAF differences as the absolute value of the difference between BAFs of anti-H3Ac and anti-H3M ChIP material. Plotting 21-SNP moving window averages revealed peaks where the two homologues exhibited long-range differences in chromatin structure. About 105 Mb of one colorectal cancer line was subject to strong allelic bias in this respect, with the longest stretch comprising 10 Mb (Di Paola et al., in preparation).

To determine whether closed conformation corresponded with transcriptional inactivation, we selected a number of genes in regions of this strong allelic bias for which there were heterozygous expressed SNPs (cSNPs). All were expressed monoallelically from the allele which was in the open conformation, indicating that, as expected, chromatin in the closed conformation was transcriptionally silent.

THE SPREADING OF CLOSED CHROMATIN CONFORMATION IN CANCER CELLS

The results of this work led to an important insight into the cancer genome. We found many megabase-plus regions of relatively uniform condensed chromatin conformation and examination of the boundaries of these domains strongly suggested that spreading of the inactive, closed conformation frequently occurred in the cancer cell lines; thus, in comparison with the normal cells, a low log R value for the anti-H3Ac ChIP curve continues into a chromatin domain which in the normal cells is open, with the mirror-image pattern being observed in the anti-H3M ChIP curve. It is arguable that since the intestine-derived cells we used for comparison are an imperfect substitute for the cancer cell lines' authentic normal cell(s) of origin the difference in extent of the closed domain did not reflect spreading but rather was a faithful reflection of the pattern inherited from the original normal precursor. However, in several instances a peak in BAF differences in a cancer line coincided with the boundary between open and closed chromatin. Since allelic conformation differences on this scale are unknown in any normal cells, the simplest interpretation is that in the cancer-derived line the extent of spreading of the closed conformation into the formerly open domain differs between the two homologues and this is inconsistent with the scenario that domains were maintained as inherited from the normal precursor. We conclude that domain spreading is generally responsible for the observed extension of the low log R values of the ChIP anti-H3Ac data. The schematic in Figure 1 illustrates this spreading and how it may affect gene expression.

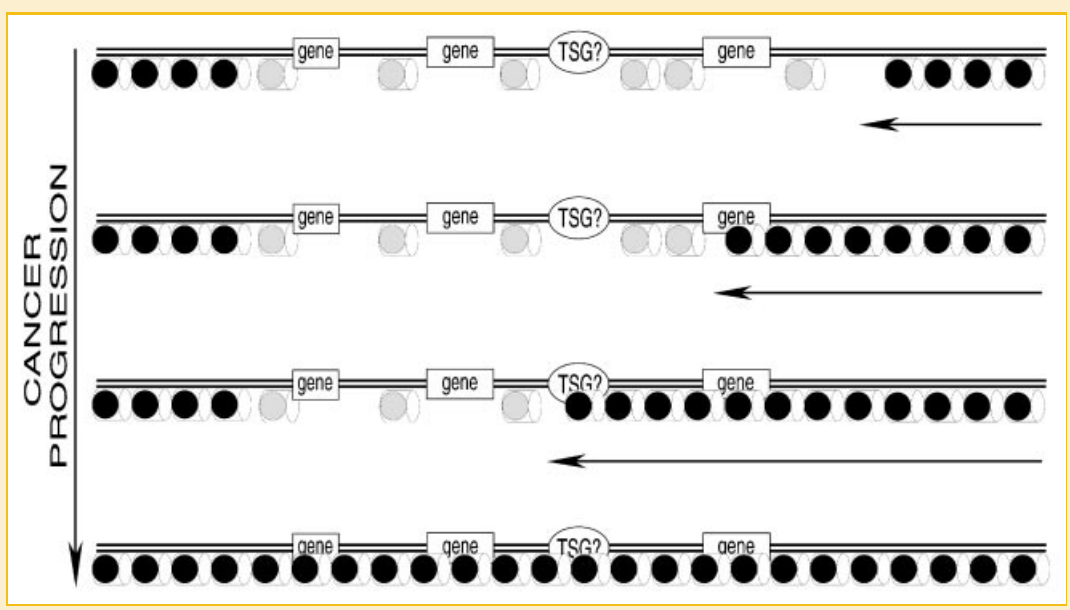

Fig. 1. A schematic of a domain of several Mb with four genes, with gene deserts flanking on either side. In this example, one of the genes may be a TSG, but similar events occur when none of the genes have suppressive function. In the normal cell (top), chromatin conformation is open through most or all of the gene-bearing domain, and closed on either flank, where trimethylated H3K9 (dark nucleosomes) predominate over acetylated H3K9 (light gray). Results of ChIP experiments described in the text reveal a strong tendency in colon cancer cells for spreading of closed conformation into the open domains; the spreading can be variable, but when none of the genes in the normally open domain (top) are essential for the cancer cell's survival and growth, the entire domain usually acquires a closed conformation in cancer, as depicted in the bottom scenario. In these cases, as discussed in the text, it is difficult to distinguish between a putative TSG and "passengers."

A variation on this theme was seen in many stretches of the genome where a limited number of expressed genes are scattered over a long domain which is otherwise in a closed conformation in the normal cells. This results in peaks rising from the "valley floor" of low log R values in the anti-H3Ac ChIP material, with complementary troughs in the anti-H3M ChIP curves. In many of these the entire domain has acquired a closed conformation in the cancer cell line, illustrated in the bottom example of Figure 1. An important point is that when a megabase-scale domain was found to be inactivated in a cancer cell line, those islands of formerly open chromatin were usually associated with either no known gene (coding or non-coding) or with genes whose role in tumor suppression is not obvious. Hundreds of gene-poor regions of between 0.5 and 3 Mb in length are scattered throughout the genome, and examples of both of the above spreading effects were seen repeatedly in the cancer genomes we analyzed (Di Paola et al., in preparation). It is tempting to speculate that there may be an economy of some sort realized by the cancer cells if a continuous domain can be rendered as heterochromatin without the need for maintaining isolated open islands of euchromatin. If this is shown to be the case, we propose that the selective advantage enjoyed by the cancer cell resulting from spreading inactivation may derive at least partially from this hypothetical economy rather than solely from silencing the genes involved.

IMPLICATIONS FOR TSG DISCOVERY

What are the lessons to be learned from these results? It is still premature to make any conclusive statement, since our results must be repeated in a variety of systems; however, we contend that the pattern of spreading of inactivation from long silent (and frequently gene-poor) domains into regions maintained in an open configuration in normal cells will be shown to be a hallmark of cancer. As such we may expect many instances of gene inactivation simply through "collateral damage"—the tumor finds a selective advantage through closing a given stretch of chromatin, and the gene happens to be situated there, simply as a passenger.

This may be the case in chromosome 2q14.2, the domain described by Frigola et al. [2006] as undergoing coordinated inactivation in colon cancer. This region is flanked on each side by gene-poor domains and thus is similar to the situation depicted in Figure 1. There may or may not be a genuine TSG in this domain, but if the tendency towards complete inactivation of a domain as a cancer progresses is real, we may observe the long-range silencing effect in this whole chromosome band, independent of any suppressive function of genes in the affected region. The driving force in favor of inactivation may not derive from turning off a TSG but from the hypothetical economy realized from establishing a uniform heterochromatic domain as discussed above.

REFERENCES

Asakawa S, Hattori N, Shimizu A, Shimizu Y, Minoshima S, Mizuno Y, Shimizu N. 2009. Analysis of eighteen deletion breakpoints in the parkin gene. Biochem Biophys Res Commun 389: 181–186.

Blauw HM, Veldink JH, van Es MA, van Vught PW, Saris CG, van der Zwaag B, Franke L, Burbach JP, Wokke JH, Ophoff RA, van den Berg LH. 2008. Copy-number variation in sporadic amyotrophic lateral sclerosis: A genome-wide screen. Lancet Neurol 7: 319–326.

Bradley WEC. 1983. Mutation at autosomal loci of Chinese hamster ovary cells: Involvement of a high-frequency event silencing two linked alleles. Mol Cell Biol 3: 1172–1181.

Bradley WEC, Raelson JV, Dubois DY, Godin E, Fournier H, Privé C, Allard R, Pinchuk V, Lapalme M, Paulussen RJA, Belouchi A. 2010. Hotspots of large rare deletions in the human genome. PLoS One 5: e9401.

Davison EJ, Tarpey PS, Fiegler H, Tomlinson IP, Carter NP. 2005. Deletion at chromosome band 20p12.1 in colorectal cancer revealed by high resolution array comparative genomic hybridization. Genes Chromosomes Cancer 44: 384–391.

Dehan E, Ben-Dor A, Liao W, Lipson D, Frimer H, Rienstein S, Simansky D, Krupsky M, Yaron P, Friedman E, Rechavi G, Perlman M, Aviram-Goldring A, Izraeli S, Bittner M, Yakhini Z, Kaminski N. 2007. Chromosomal aberrations and gene expression profiles in non-small cell lung cancer. Lung Cancer 56: 175–184.

Di Paola D, Rampakakis E, Zannis-Hadlopoulos M, Bradley WEC. In preparation. Megabase-scale spreading og epigenetic inactivation in cancer cells.

Dobrovic A, Gareau JL, Ouellette G, Bradley WEC. 1988. DNA methylation and genetic inactivation at thymidine kinase locus: Two different mechanisms for silencing autosomal genes. Somat Cell Mol Genet 14: 55–68.

Frigola J, Song J, Stirzaker C, Hinshelwood RA, Peinado MA, Clark SJ. 2006. Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. Nat Genet 38: 540–549.

Grant SG, Campbell CE, Duff C, Toth SL, Worton RG. 1989. Gene inactivation as a mechanism for the expression of recessive phenotypes. Am J Hum Genet 45: 619–634.

Hesson LB, Cooper WN, Latif F. 2007. Evaluation of the 3p21.3 tumour-suppressor gene cluster. Oncogene 26: 7283–7301.

Lerman MI, Minna JD. 2000. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: Identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. Cancer Res 60: 6116–6133.

Novak P, Jensen T, Oshiro MM, Watts GS, Kim CJ, Futscher BW. 2008. Agglomerative epigenetic aberrations are a common event in human breast cancer. Cancer Res. 68: 8616–8625.

Picchio MC, Martin ES, Cesari R, Calin GA, Yendamuri S, Kuroki T, Pentimalli F, Sarti M, Yoder K, Kaiser LR, Fishel R, Croce CM. 2004. Alterations of the tumor suppressor gene Parkin in non-small cell lung cancer. Clin Cancer Res 10: 2720–2724.

Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin ML, Ordóñez GR, Bignell GR, Ye K, Alipaz J, Bauer MJ, Beare D, Butler A, Carter RJ, Chen L, Cox AJ, Edkins S, Kokko-Gonzales PI, Gormley NA, Grocock RJ, Haudenschild CD, Hims MM, James T, Jia M, Kingsbury Z, Leroy C, Marshall J, Menzies A, Mudie LJ, Ning Z, Royce T, Schulz-Trieglaff OB, Spiridou A, Stebbings LA, Szajkowski L, Teague J, Williamson D, Chin L, Ross MT, Campbell PJ, Bentley DR, Futreal PA, Stratton MR. 2010a. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 463: 191–196.

Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, Lin ML, Beare D, Lau KW, Greenman C, Varela I, Nik-Zainal S, Davies HR, Ordoñez GR, Mudie LJ, Latimer C, Edkins S, Stebbings L, Chen L, Jia M, Leroy C, Marshall J, Menzies A, Butler A, Teague JW, Mangion J, Sun YA, McLaughlin SF, Peckham HE, Tsung EF, Costa GL, Lee CC, Minna JD, Gazdar A, Birney E, Rhodes MD, McKernan KJ, Stratton MR, Futreal PA, Campbell PJ.

2010b. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 463: 184–190.

Sironi M, Pozzoli U, Comi GP, Riva S, Bordoni A, Bresolin N, Nag DK. 2006. A region in the dystrophin gene major hot spot harbors a cluster of deletion breakpoints and generates double-strand breaks in yeast. FASEB J 20: 1910–1912.

Smith JS, Costello JF. 2006. A broad band of silence. Nat Genet 38: 504–506.

Suzuki MM, Bird A. 2008. DNA methylation landscapes: Provocative insights from epigenomics. Nat Rev Genet 9: 465–476.

Wang F, Denison S, Lai JP, Philips LA, Montoya D, Kock N, Schüle B, Klein C, Shridhar V, Roberts LR, Smith DI. 2004. Parkin gene alterations in hepatocellular carcinoma. Genes Chromosomes Cancer 40: 85–96.

Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. 2007. The genomic landscapes of human breast and colorectal cancers. Science 318: 1108–1113.