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# Review

# Gene Dysregulations Driven by Somatic Copy Number Aberrations-Biological and Clinical Implications in Colon Tumors

## *A Paper from the 2009 William Beaumont Hospital Symposium on Molecular Pathology*

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**The majority of colorectal cancer (CRC) cases have chromosomal instability, in which the tumor genome is characterized by gross chromosomal aberrations such as gains in 20q, 13q, 8q, and 7, and losses in 4, 8p, 18q, and 17p. These somatic copy number changes (gains, losses, and somatic uniparental disomies) are crucial to CRC progression as they drive genes toward cancer-promoting (oncogenic or tumor suppressive) states. Numerous studies have shown that the loss of 18q or 8p is associated with poorer clinical outcome in CRCs. Either chromosomal arm may contain a tumor suppressor gene (or genes), whose deactivation by copy loss (loss of wild-type allele, decreased expression) can be crucial to the later stages of cancer progression. Our own integrated genomic analysis (single nucleotide polymorphism array, expression array) of more than 200 CRC tumor and normal samples indicates that the overall down-regulation of genes within the 8p or 18q arm is associated with lower survival rate. Among the often down-regulated, poor prognosis-associated 8p genes is *MTUS1*, whose gene product (a mitotic spindle-associated protein) was recently demonstrated to have a tumor suppressive property. Within 18q is *ATP5A1*, which codes for the catalytic a component of mitochondrial $H^+$-ATP synthase. Like *SMAD4* (also in 18q), the decreased expression of *ATP5A1* appears to be a marker of unfavorable clinical outcome in CRCs. *(J Mol Diagn 2010, 12:552–561; DOI: 10.2353/jmoldx.2010.100098)***

Colorectal cancer (CRC) is generally associated with more affluent nations. However, recent statistics indicate that the incidence rate of CRC in the developing world from the 1980s until the first few years of this millennium has increased significantly,[1] and the disease is turning into a major global health care burden.[2] The main culprit may be the growing popularity of Western diet (ie, high in proteins and fats, low in fibers and vegetables) across the globe. In the United States, more than 150,000 people are diagnosed with the disease each year, resulting in over 50,000 deaths.[3] In addition to environmental factors (ie, diet, cigarette smoking, and alcohol consumption), genetics can also contribute to CRC incidence. Fewer than 10% of CRC cases may arise from highly-penetrant inherited mutations (eg, mutations in the *APC* gene for familial adenomatous polyposis or FAP cases and mutations in mismatch repair genes for hereditary nonpolyposis colorectal cancer or HNPCC cases).[4] Results from recent genome-wide association studies also indicate that even spontaneous cases (about 70% of CRC cases) may also be influenced by low penetrant predisposition single nucleotide polymorphisms (SNPs).[5]

CRC cases have two major types of genomic instability. Most CRCs (including FAP cases) are characterized by gross chromosomal aberrations, such as losses or gains of whole or partial chromosomes or chromosomal arms, and are therefore classified as chromosomal insta-

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bility (CIN) tumors.[6] The rest (~16%[7]) are classified as microsatellite instability (MIN). MIN tumors are mainly diploid and have a mutator phenotype because of defective mismatch repair. The defective mismatch repair (which may be due to an inherited mutation or promoter hypermethylation of an mismatch repair gene)[8,9] results in replication errors within the microsatellite markers. This distinction between MIN and CIN tumors actually has clinical implications since the former is usually associated with better prognosis.[10,11]

## *Analytical Tools Used To Detect Somatic Copy Number Aberrations in Cancer (Including CRCs)*

The field of cancer cytogenetics may have started when Theodor Boveri and David Hansemann published their seminal works between the late nineteenth and early twentieth centuries.[12] Essentially, these two pioneers hypothesized a link between carcinogenesis and abnormal constitution of the chromosomes. The discovery of correct number of human chromosomes by Tjio and Levan in 1956[13] was shortly followed by karyotypic characterizations of various types of cancer. In the 1990s, fluorescence *in situ* hybridization became a widely used technique for detecting chromosomal aberrations (gains or losses at specific chromosomal regions) in tumor cells examined in either metaphase or interphase state.[14] At around the same time, investigators started the routine use of PCR-based microsatellite marker analysis to identify loss of heterozygosity (LOH) in certain chromosomal regions.[15] Comparative genomic hybridization (CGH) (wherein tumor and normal genomic DNAs are differentially labeled and co-hybridized onto a metaphase chromosome of normal karyotype) was also introduced in the early 1990s and became a popular technique in genome-wide chromosomal characterization of tumors.[16] CGH was then further developed into an array format capable of detecting chromosomal aberrations at a much higher resolution (10-kb range).[17] A very significant percentage of publications on cancer cytogenetics from the mid-1990s through the last decade were reports of data generated from CGH and CGH arrays (more than 3000 articles retrieved on entering the key words "cancer" and "comparative genomic hybridization" in a PubMed search).

Another important breakthrough in molecular cancer cytogenetics was the introduction of SNP arrays.[18] Like

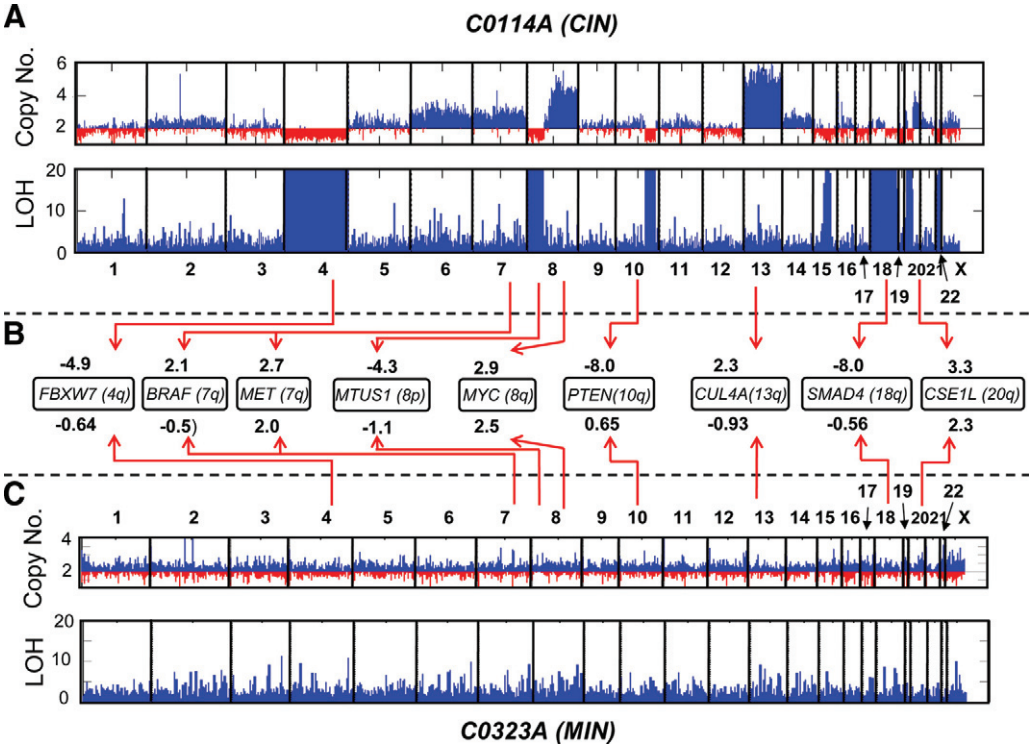


**Figure 1.** Cytogenetic characterization of CIN (primary tumor sample C0114A; **A**) and MIN (primary tumor sample C0323A; **C**) CRC genomes by Affymetrix Xba 240 50K SNP array analysis. As shown here, as well as previously,[21] C0114A has acquired copy number losses in chromosomes 4, 22, 8p, and 20p, and gains in chromosomes 7, 8q, 13, and 20q. Also lost were significant portions of 18q and 10q. Unlike C0114A, C0323A did not have noticeable copy number aberrations. **B** is a comparison of the expression levels of some of the known oncogenes and tumor suppressor genes between the two tumor samples. For example, the expression level (z) of the tumor suppressor gene *SMAD4* in C0114A and C0323A (relative to normal colon) are −8.0 and −0.56, respectively. This suggests that the loss of chromosome 18q contributed to C0114A's low mRNA level for *SMAD4*. Compared with C0323A, C0114A also exhibited lower expression levels for the tumor suppressor genes *FBXW7*, *PTEN*, and *MTUS1*, which are all located in chromosomal regions lost in C0114A. The oncogenes *MYC*, *MET*, *BRAF*, and *CUL4A* are all located in regions of gain in the C0114A genome. This may explain why C0114A has relatively higher expression levels (at varying degrees) of these genes. $z = (I_t - \bar{I}_n)/\sigma_n$; where $I_t$ is the normalized, log transformed intensity value (I) of the Affymetrix U133A probe set for the tumor sample, while $\bar{I}_n$ and $\sigma_n$ are the average and SD (respectively) of the I values for 53 normal colon samples. For each gene represented by multiple probe sets (*PTEN,CSE1L, MET,MTUS1, CUL4A, SMAD4*), the z value indicated in **B** is actually the average for all probe sets representing the gene. Note: Each genome-wide scan includes a copy number chart (baseline copy number is 2) and LOH chart. High LOH values (for the charts, the LOH value is capped at 20), indicated by tall blue bars represent segments in the chromosome of contiguous homozygous SNPs. Regions of copy loss usually correspond to regions of high LOH.

CGH arrays, SNP arrays can be used for high-resolution (albeit more complicated) copy number analysis of cancer genomes. Because of its capability to read SNP copy number and genotype simultaneously, a SNP array can detect a chromosomal region with somatic uniparental disomy (UPD; copy loss LOH followed by the duplication of remaining chromosomal segment), now found to occur frequently in neoplastic transformation.[19] In recent years, both CGH array (the oligonucleotide-based platform in particular) and SNP array have become the standard techniques for genome-wide characterization of chromosomal aberrations in various cancer types. Neither of these techniques require metaphase cells, and with their high probe densities (almost two million SNP plus copy number probe sets in the Affymetrix SNP Array 6), have the power to detect very narrow regions of deletions and gains (can be less than 10 kb in size). For the comprehensive comparisons of various cytogenetic techniques used in cancer research, see a recent review by Maciejewski et al.[20]

## Somatic Copy Number Aberrations in CRCs

The genome-wide chromosomal scan of a typical CIN CRC is shown in Figure 1A (generated by SNP array analysis). The copy number changes in this tumor sample (C0114A) include losses in chromosomes 4, 8p, and 18, as well as gains in 7, 8q, 13q, and 20q[21]. Along with losses in 14q, 17p, and 15q, these are the most commonly occurring chromosomal aberrations in CRCs.[22–25] In contrast, a typical MIN tumor (sample C0323A; Figure 1C is devoid of these chromosomal aberrations and remains diploid. Of all of the common somatic copy number changes in CRCs, it is the loss of 18q[24,26–39] that is most clearly associated with poor prognosis (Table 1). This conclusion, which is derived from numerous studies that used varying techniques (microsatellite analysis, CGH, CGH array, SNP array, karyotyping), is actually consistent with the observation that 18q loss is also associated with distant metastasis.[40] As indicated in Table 1,[24,26] the loss of 8p[24,34–39,41] or 17p (the location of *TP53* tumor suppressor gene)[33,34,42] is also linked to lower survival rate.

## How Copy Number Aberrations Lead to Dysregulation of Genes Crucial to CRC Progression

Somatic chromosomal copy number changes can confer selective advantages to proliferating cancer cells because these aberrations lead to dysregulation of genes that are important in carcinogenesis (Figure 2, A and B).

### Dysregulation of Genes in Regions of Chromosomal Gain

In theory, a copy number gain (either interstitial, partial arm, whole arm, or whole chromosome) will result in the elevated expression of its resident genes (Figure 2A). For this reason, we can imagine that within the regions of somatic copy gains are genes whose increased transcriptional level can contribute to the process of cancer progression (see Path A1 in Figure 2A). Examples include the proto-oncogenes *MYC* (transcription factor)[43] and *MET* (receptor tyrosine kinase),[44] located in the often gained 8q and 7q arms, respectively. Both these genes are often up-regulated in CRCs.[45–47] The 13q and 20q chromosomal arms, both of which harbor a great percentage of genes with up-regulated expression,[24,25] are also often gained in CRCs. In our CRC genome-wide

**Table 1.** Studies Which Demonstrated That Certain Chromosomal Aberrations Are Good Prognostic Markers in Colorectal Cancer (Arranged Chronologically)

| Year | Poorer prognosis correlated to | Total number of samples analyzed | Analysis by | Reference no. |
|---|---|---|---|---|
| 1994 | Loss in 18q | 145 (Stages II, III; no HNPCC) | MS markers (18q) | 26 |
| 1997 | Loss in 17p | 141 (had undergone liver resection) | MS markers (5q, 8p, 10q, 15q, 17p, 18p, and 18q) | 42 |
| 1998 | Loss in 18q | 151 (had undergone surgery) | MS markers (18q) | 27 |
| 1998 | Loss in 18q | 125 (no HNPCC) | MS markers (18q) | 28 |
| 1998 | Loss in 18q | 118 (Stages II, III; had undergone surgery) | MS markers (18q) | 29 |
| 1999 | Loss in 18q21 | 195 | MS markers (18q21) | 30 |
| 1999 | Loss in 8p | 508 CRC patients (Stages B2, C) | MS markers (5q, 8p, 15q, 17p, 18q) | 41 |
| 2001 | Losses in 18q, 14q, 8p, 4q, 1p; gain in 20q | 67 (Stages A, B, C, D) | CGH | 31 |
| 2001 | Loss in 18q | 460 (Stage II, III; treated with various combinations of fluorouracil and leucovorin) | MS markers (18q, 17p, and 8p) | 32 |
| 2002 | Losses in 18q, 17p | 228 | MS markers (18q, 17p, and 5q) | 33 |
| 2002 | Losses in 18q, 17p, 8p | 168 (Stage III) | MS markers (to 3p, 4p, 5q, 8p, 9p, 13q, 17p, and 18q) | 34 |
| 2002 | Losses in 18q, 8p | 180 | Digital SNP analysis (8p and 18q) | 35 |
| 2004 | Losses in 18q, 8p | 123 (Stage II, III; had undergone curative resection) | MS markers (18q, 8p, and 4p) | 36 |
| 2004 | Loss in 18q; aberration in 8 | 150 (Stage II, III) | Karyotyping | 37 |
| 2006 | Losses in 18q, 8p, 4p | 70 (had undergone surgery, most without chemotherapy) | Array CGH | 38 |
| 2007 | Losses in 18q12-qter, 8p12-pter; gain in 8q23 and 8q24 | 73 (Stage I, II, III, IV) | CGH | 39 |
| 2009 | Losses in 18q, 15q, 8p, 4p | 182 (Stage I, II, III, IV ) | SNP array | 24 |

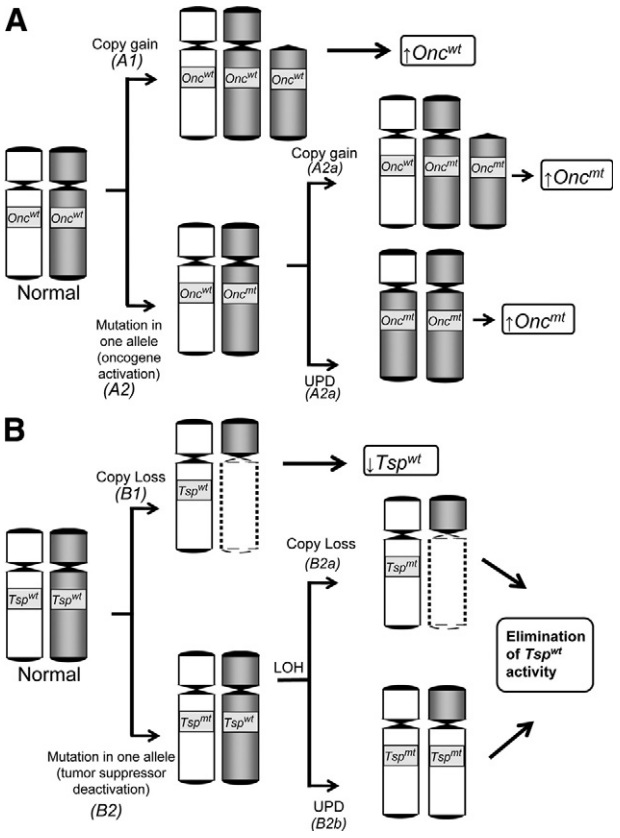


**Figure 2.** Copy number gain (**A**) or loss (**B**) contributes to the dysregulation of an oncogene (*Onc*) or tumor suppressor gene (*Tsp*) toward tumor promotion. wt, wild-type; mt, mutant; ↑, higher expression level; ↓, lower expression level.

expression analysis, one of the 13q genes that turned out to be very highly up-regulated was *CUL4A*, which has also been found to be copy number-dysregulated (ie, amplified in both copy number and expression) in breast cancer.[48], CUL4A is as an important component of an ubiquitin ligase system involved in proteasomal degradation of the tumor suppressor protein $p27^{KIP1}$.[49] Within the often gained 20q arm is *CSE1L* (coding for a cellular apoptosis susceptibility protein), whose overexpression was recently shown to enhance the invasive potential of cancer cells.[50] This is consistent with another recent report of direct correlation between CSE1L expression and lymphatic metastasis among CRC patients.[51] In addition, we identified CRC samples with amplifications at narrow regions of chromosomes. One particular sample acquired an amplification (>4 copies) in the region of the 6p arm (which is not commonly gained in CRCs) covering the locus of the oncogene *VEGF* (unpublished data). Not surprisingly, this sample also registered one of the highest VEGF expression level among our CRC samples. The VEGF overexpression of this tumor sample may have been relevant clinically, since the protein is the direct target of bevacizumab, which in combination with the FOLFOX4 is already an FDA-approved regimen to treat metastatic CRCs.[52]

Certain CRC-associated proto-oncogenes such as *KRAS*,[53] *BRAF*,[54] and *PIK3CA*,[55] usually need mutational activation (Path A2 in Figure 2A) to be oncogenic. For these aforementioned genes (KRAS is an effector molecule for both BRAF and PIK3CA[56]), monoallelic mutation without the accompanying copy number increase may suffice due to the dominant nature of the mutation.[57] However, a recent report by Soh and colleagues[57] demonstrated that a considerable percentage (6/60; 10%) of CRC tumor samples have acquired simultaneous *KRAS* mutations and copy gains. In our own analysis (unpublished results), we found five of 74 CRC tumor samples (7%) having simultaneous *KRAS* mutation and copy gain. In theory, the cancer- promoting activity of a mutation-activated oncogene can be further enhanced by gaining an additional copy of the mutated allele (Path A2a in Figure 2A). Alternatively, the oncogenic activity of a mutation-activated oncogene may also be elevated by somatic UPD (Path A2b in Figure 2A). In the same report, Soh and co-workers[57] presented evidence that lung adenocarcinoma samples (those with SNP array and sequencing data) can acquire a *KRAS* mutation and UPD at the same time. It is important to note that the *KRAS* locus is located on the 12p arm, which rarely gains additional copies in CRCs; thus *KRAS* copy number gains may just be locus-specific (the authors used quantitative PCR for copy number detection).

## *Dysregulation of Genes in Regions of Chromosomal Loss (and Somatic UPDs)*

By its simplest definition, a tumor suppressor gene codes for a protein that can derail tumor initiation or progression. The deactivation of a tumor suppressor gene is therefore crucial to carcinogenesis. Reduced expression, through copy loss, is one mechanism this can be achieved (Figure 2B, Path B1). For instance, the loss of the 18q arm can result in down-regulation (as exemplified in sample C0114A; see Figure 1B) and subsequent deactivation of the tumor suppressor gene *SMAD4* (18q 21.1). An important component of the transforming growth factor-$\beta$ signaling pathway, SMAD4 forms a complex with phosphorylated SMAD2 and SMAD3. The complex then translocates to the nucleus[58] to promote the transcription of genes involved in growth inhibition, such as the cyclin-dependent kinase inhibitors $P15^{INK4b}$ (*CDKN2B*) and $P21^{CIP1}$(*CDKN1A*).[59] The SMAD complex may also be involved in the transcriptional regulation of genes that are relevant to cell invasion and metastasis, including *MMP9* (matrix metalloproteinase-9),[60] which is consistent with SMAD4 down-regulation having been associated with CRC metastasis[61] and its poorer clinical outcome.[62] Also within the 18q arm is the gene *DCC* (coding for a netrin-1 receptor), initially identified as a tumor suppressor gene[63] and for a long time was considered the primary reason why 1oss of 18q correlates to poor prognosis in CRC.[64] Recently, DCC has been shown to induce apoptosis conditionally (ie, when it is not engaged by its ligand Netrin-1) and thus may have some tumor suppressor functionality.[65] The analysis of our CRC expression data showed that another frequently down-regulated 18q gene is *ATP5A1*, which codes for a protein that forms the catalytic subunit of mitochondrial $H^+$-ATP

synthase.[66] Several groups have described CRC samples exhibiting reduced activity of this enzyme, presumably leading to mitochondrial dysfunction[67,68] and thus an elevated rate of glycolysis, which according to Warburg's hypothesis characterizes tumor cells.[69] Another study has shown that CRC's resistance to 5-flurouracil (the standard chemotherapeutic drug against CRC) correlated with down-regulation of mitochondrial $H^+$-ATP synthase.[70]

Chromosome 4, the location of the tumor suppressor gene *FBXW7* (or *CDC4*), which has been shown to be down-regulated in CRCs, is also often lost in CRCs.[71] FBXW7 mediates the proteolysis of several oncoproteins and thus may have important role in cancer progression.[72] Another recurrent CRC chromosomal aberration is the loss of 17p. This chromosomal arm includes the locus for *MAP2K4*, a gene with a proven role in metastasis suppression through the p38 and JNK pathways.[73] It is not surprising then that our own analysis revealed that *MAP2K4* is one of the most highly down-regulated genes within the frequently lost 17p arm. Among the genes we found to be underexpressed in CRCs because of 8p loss is *MTUS1* (the down-regulation of this gene in CRCs has also been reported by another group[74]). *MTUS1* codes for a mitotic spindle-associated protein shown to be involved in the eventual reduction of mitotic rate.[75]

Somatic copy number losses may also occur in narrower regions (may be as narrow as the gene locus) of the chromosomes. We have identified several cases with small deletions in a 10q region, which includes the *PTEN* locus. The tumor suppressor function of PTEN, a negative regulator of PI3K in the AKT signaling pathway, is well described in the literature.[76] Another tumor suppressor gene that is found to exhibit locus-specific LOH is *KLF6* (Kruppel-like factor 6) within 10p15 region.[77] The transcription factor *KLF6* is frequently underexpressed in CRCs.[78]

In essence, LOH results in the reduced expression of a fully functioning (wild-type) tumor suppressor gene product. For many tumor suppressor genes, however, complete deactivation can be achieved if LOH is preceded by a mutation in one allele (see Path B2 in Figure 2B). This type of gene dysregulation can be observed in the tumor suppressor genes *APC* and *TP53*,[79] which are among the two most highly mutated genes in CRCs.[80] In such cases, LOH is the "second hit" to an earlier somatic mutation ("first hit") in a manner that is consistent with Knudson's hypothesis.[81] In CRCs, the loss of a *TP53* allele can result from the loss of the entire 17p arm. Loss of the 5q arm (where *APC* locus is located) also occurs in CRC samples, but not as common as the losses of 18q, 8p, or 17p.[22] As shown in Path B2b (in Figure 2B), LOH may also be acquired through somatic UPD. With the use of SNP arrays, we have identified a case in which the mutated *APC* gene had acquired a somatic UPD.[19] Aside from *TP53* and *APC*, two other tumor suppressor genes located in regions of frequent loss and found to be mutated in CRCs are *SMAD4* (18q) and *FBXW7* (4q).[80,82,83]

## *Integrated Analyses of SNP Array, Expression Array, and Clinical Data Can Identify Prognosis-Associated Genes in Regions of Chromosomal Aberrations*

Results from our combined genome-wide expression and molecular cytogenetic analyses point to the up-regulation or down-regulation of a sizeable number of genes[25,84]

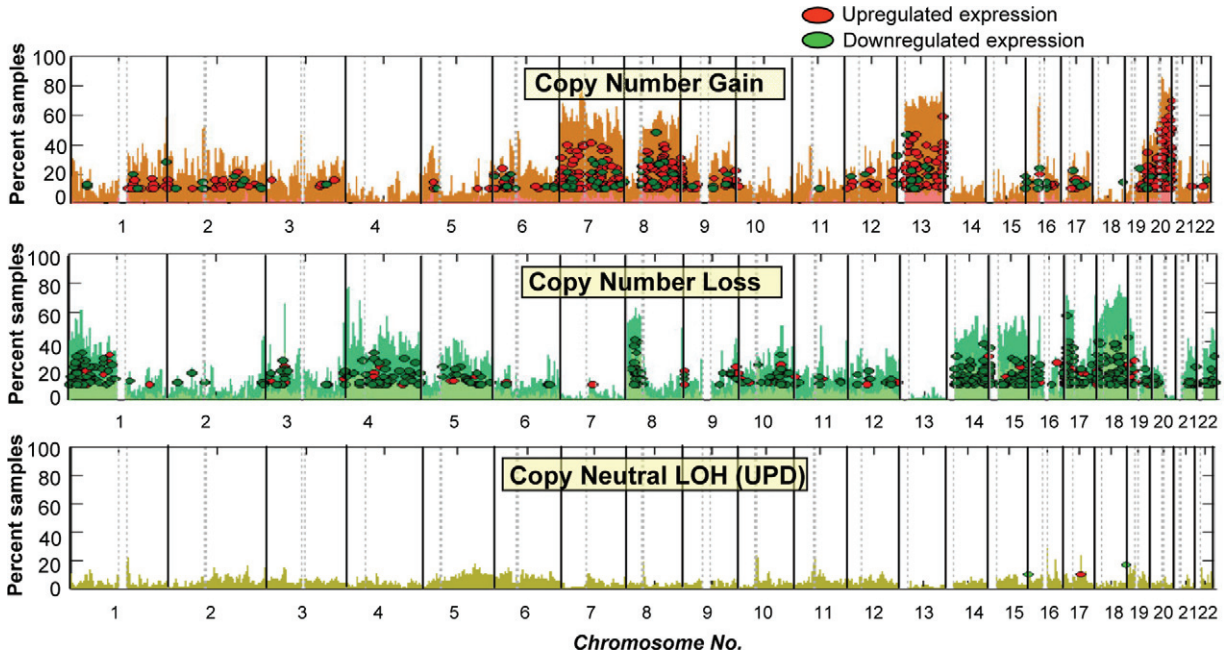


**Figure 3.** Overall view of gene expression dysregulations within chromosomal aberrations. Shown are the percentages of samples with gains (**top chart**), losses (**middle chart**), and copy neutral-LOH or somatic UPDs (**bottom chart**) in every autosomal chromosome. Each ellipse refers to a U133A probe set representing a gene located in the region of aberration with $z \geq 3$ (up-regulated), or $z \leq -3$ (down-regulated) in at least 10% of the CRC samples. Note that chromosomal gains and losses are populated by numerous genes that are up-regulated and down-regulated, respectively.

located within the chromosomal regions of gain or loss, respectively (Figure 3). Extensive literature search allows us to make some intelligent suppositions as to which genes within these aberrant arms can possibly contribute to cancer progression and are therefore worthy of further biological studies. If the tumor samples have accompanying clinical records, we can further classify these copy number-dependent genes based on how their expression levels correlate to prognosis. Using this approach, we found that the genes *MTUS1, ADAMDEC1* (member of the disintegrin metalloproteinase family of genes), *EPHX2* (member of the epoxide hydrolase family), and *PPP2CB* (catalytic subunit of phosphatase 2A) are among the down-regulated 8p genes whose lower expression level correlated with poorer prognosis (Figure 4, A and C). As stated above, *MTUS1* is most likely a tumor suppressor gene.[74,75,85] The down-regulation of *ADAMDEC1* and *EPHX2* were recently associated with colon cancer metastasis,[86] and *PPP2CB* codes for the catalytic component of tumor suppressor protein phosphatase 2A.[87] We now hypothesize that the poor CRC prognostication of 8p loss may be explained by the fact that it harbors a number of genes with tumor suppressive properties and which play crucial roles in later stages of carcinogenesis. Likewise, our integrated genome-wide molecular profiling and clinical data analyses indicate that there are 18q genes other than *SMAD4* (and possibly *DCC*) whose lower expression levels are indicative of worse clinical outcome (Figure 4, B and C). One of these is *ATP5A1,* whose biological function is discussed above. Another possibly relevant gene within the 18q arm is *NEDD4L* (its down-regulation also correlating to poor prognosis based on our analysis), whose decreasing expression level correlated to increasing Gleason score (a measure of aggressiveness) in prostate cancer.[88]

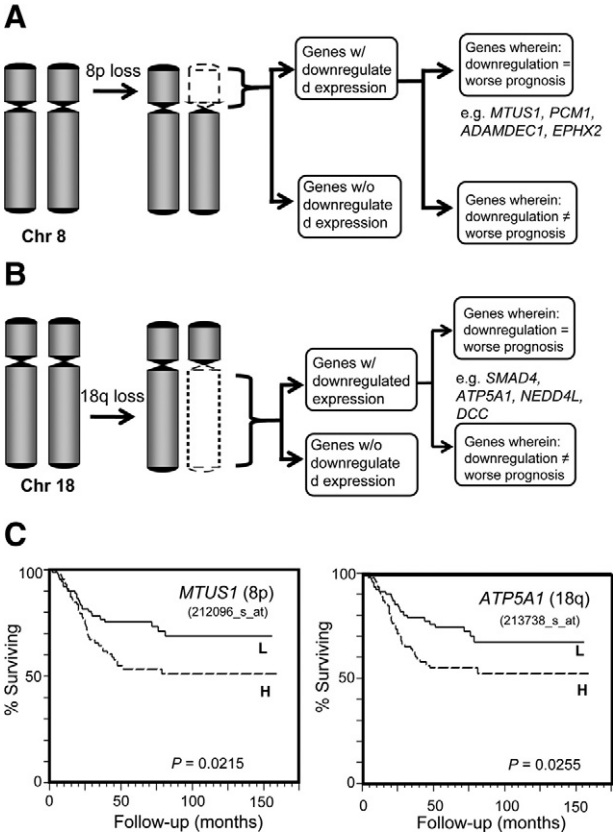


**Figure 4.** The loss of the 8p (**A**) or 18q (**B**) chromosomal arm has been frequently associated with poorer prognosis in CRCs. Our combined SNP and expression array analyses (more than 200 tumor samples) have demonstrated that not all genes within these lost arms have down-regulated expression. A subset of genes with down-regulated expression appear to have prognostic relevance (ie, down-regulation = poor prognosis), including *MTUS1, PCM1, ADAMDEC1,* and *EPHX2* in 8p and *ATP5A1, SMAD4, NEDD4L,* and *DCC* in 18q. **C:** Kaplan-Meier plots based on the expression levels of *MTUS1* and *ATP5A1* among the primary tumors of 182 CRC patients. In the Kaplan-Meier analysis, the CRC patients were divided into low (L) and high (H) expression groups. Also indicated is the log rank *P* value for each gene.

## *Beyond Somatic Copy Number Aberrations: Other Factors That Influence Cancer Gene Dysregulations in Sporadic CRCs*

Somatic copy number changes and UPDs are not the only types of chromosomal aberrations a normal cell acquires to dysregulate genes toward tumor promoting activities. Chromosomal translocations (interchange of parts between non-homologous chromosomes) can lead to formation of fusion proteins with oncogenic properties or overexpression of existing proto-oncogenes (eg, when *MYC* is positioned closer to an enhancer/promoter element of another gene such as *IgH*).[89] Although commonly identified with hematological neoplasms (such as leukemias and lymphomas), a fusion oncogene [*EML4-ALK* (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase)] was recently detected in CRC tumors with the use of exon array profiling.[90] Promoter hypo/hypermethylation can also dysregulate the expression of cancer-related genes such as *MGMT* (*O*-6-methylguanine-DNA methyltransferase), *RARB* (retinoic acid receptor β) and *CDKN2A* (cyclin-dependent kinase inhibitor 2A).[91,92] In our integrated SNP array/expression array

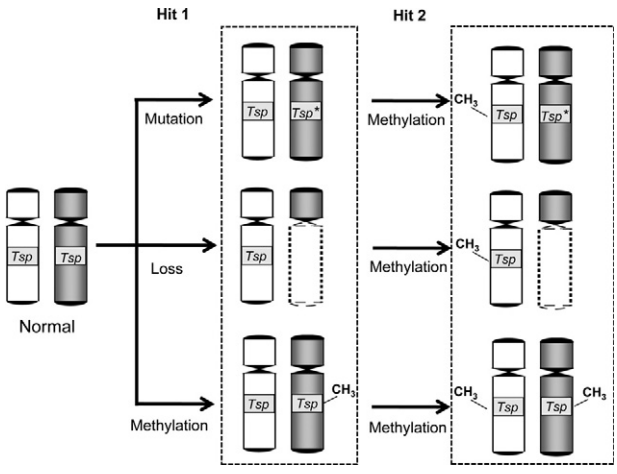


**Figure 5.** How promoter hypermethylation contributes to dysregulation of a tumor suppressor gene (*Tsp*). As shown in this diagram, one of the wild-type *Tsp* alleles of a normal cell gets deactivated (Hit 1) through mutation (*Tsp**), copy loss, or promoter hypermethylation. It may then be necessary to deactivate the second allele (Hit 2) for the *Tsp* to attain its tumor promoting state. As shown in this model, the deactivation of the second allele may also be attained by promoter hypermethylation.

analyses of mostly CIN tumors, we have also identified genes whose up-regulation/down-regulation are not related to copy number changes, thus their changes in expression levels may be epigenetically regulated. One of these genes is *CDH3* (P-cadherin), which is located in chromosome 16 and found to be highly expressed in 77% of CRC samples. A recent study has shown that CDH3's overexpression in CRC occurs largely through hypomethylation of CpG sites at its promoter region.[93] In theory, a promoter hypermethylation of a tumor suppressor allele can have the same result as a copy loss (ie, lower overall expression) (Figure 5).

The cancer-promoting activities of tumor suppressor genes and oncogenes may also be regulated posttranscriptionally by non-coding microRNAs, 18- to 25-nucleotide-long RNAs, which can inhibit the translation of their complementary or near-complementary mRNAs.[94] Effectively, a microRNA molecule is oncogenic or tumor suppressive if it is inhibitory to the translation of tumor suppressor or oncogene transcript, respectively. In experiments involving CRC cells, the microRNA miR-135a&b was shown to suppress APC translation (thus oncogenic),[95] whereas MYC expression can be down-regulated by microRNAs miR-43[96] and let-7.[97]

## Future Directions and Concluding Remarks

CRCs (in particular, those classified as CIN) do not just acquire certain chromosomal copy number aberrations randomly. Within regions of gains, copy losses, or UPDs are genes, which on dysregulation, are transformed into cancer-promoting states. These recurrent aberrations, which often involve a whole chromosomal arm (eg, 8p, 8q, 20q), can lead to dysregulation (usually by causing changes in expression level) of numerous genes in either their wild-type or mutated states. Eventually, a single run of next-generation sequencing will be all that is needed to acquire genome-wide mutational, copy number, and LOH profiles of a tumor sample.[98] Such a more robust set of information (in addition to genome-wide transcriptional read-out) will certainly strengthen our assessment regarding which genes within the aberrant arms may have some roles in cancer progression. Examples of genes worthy of a closer look would be a mutated, down-regulated gene in a lost region; a mutated (homozygous) gene in a somatic UPD region; and a mutated, up-regulated gene in a region of gain.

The integration of patient survival data to our genome-wide expression analysis led to our observation that among many 18q and 8p genes, lower expression level equates to poor prognosis in CRCs. This may explain why the loss of the 18q or 8p arm has been correlated to worse CRC clinical outcome. We speculate that certain copy number–dependent genes within these arms are essential to the later stages of cancer progression. The next step would be to further evaluate the cancer-related functionalities (such as possible roles in cell cancer proliferation and metastasis) of some of these candidate 8p and 18q genes. In one experimental design, the association of an 8p gene down-regulation to poor CRC prognosis can be tested *in vitro* by forcibly inhibiting the gene in a cancer cell line, which will then be exposed to a drug specifically used for CRC treatment (such as 5-flurouracil and oxaliplatin). For example, a decrease in the cancer cell-killing effect of 5-flurouracil after *PPP2CB* (an 8p gene) inhibition will be consistent with *PPP2CB* down-regulation correlating to poorer clinical outcome, and the potential use of the gene as a prognostic marker may be useful for clinical management of CRC.

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