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The consequences of chromosomal aneuploidy on the transcriptome of cancer cells☆

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Abstract

Chromosomal aneuploidies are a defining feature of carcinomas, i.e., tumors of epithelial origin. Such aneuploidies result in tumor specific genomic copy number alterations. The patterns of genomic imbalances are tumor specific, and to a certain extent specific for defined stages of tumor development. Genomic imbalances occur already in premalignant precursor lesions, i.e., before the transition to invasive disease, and their distribution is maintained in metastases, and in cell lines derived from primary tumors. These observations are consistent with the interpretation that tumor specific genomic imbalances are drivers of malignant transformation. Naturally, this precipitates the question of how such imbalances influence the expression of resident genes. A number of laboratories have systematically integrated copy number alterations with gene expression changes in primary tumors and metastases, cell lines, and experimental models of aneuploidy to address the question as to whether genomic imbalances deregulate the expression of one or few key genes, or rather affect the cancer transcriptome more globally. The majority of these studies showed that gene expression levels follow genomic copy number. Therefore, gross genomic copy number changes, including aneuploidies of entire chromosome arms and chromosomes, result in a massive deregulation of the transcriptome of cancer cells. This article is part of a Special Issue entitled: Chromatin in time and space.

Keywords

Aneuploidy; Gene expression; Copy number alterations; Solid tumors; Cell lines

1. Introduction: the landscape of chromosomal aberrations in solid tumors

The discovery of the Philadelphia chromosome in patients afflicted with chronic myelogenous leukemia (CML), and the description of a balanced translocation as its cause eliminated any doubt that cancer has a genetic basis, and is, in fact, a disease of the chromosomes [1,2]. In particular with respect to the hematological malignancies, i.e.,

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leukemias and lymphomas, this paradigm applies to many different tumor entities. The consequences of balanced chromosomal translocations are either the generation of a fusion protein, as in the case of the bcr-abl rearrangement in CML, or the deregulation of a cellular oncogene through placement of a physiologically non-expressed oncogene into the vicinity, and thus under the control, of genes with active enhancer elements; this is, for example, observed in Burkitt's lymphoma with *MYC* and *IGH* [3–5]. In both scenarios, the chromosomal translocations point to the pathognomonic genetic aberrations that are ultimately responsible for the malignant transformation of hematological cells. The fact that targeted therapies against the consequences of such fusions, e.g., imatinib in the case of CML [6], show great treatment efficacy, further supports this notion.

Most of the discoveries of chromosomal aberrations in hematological malignancies were based on the analysis of metaphase chromosomes using chromosome-banding techniques [7,8]. The application of chromosome banding to the study of solid tumors of epithelial origin, i.e., the carcinomas, turned out to be considerably more challenging [8–10]. This was partly due to the fact that it was more difficult to prepare metaphase chromosomes of adequate quality for analysis, but was also attributable to the sheer number and the complexity of cytogenetic abnormalities in solid tumors. When combined, this led to the perception that the cytogenetic profiles of solid tumors are governed by karyotypic complexity and clonal heterogeneity, induced by centrosome amplification, apolar mitoses, and ongoing chromosomal instability, which, in short, cause a cytogenetic chaos [11]. Inadvertently, this resulted in the interpretation and widespread belief that chromosomal aberrations in carcinomas, in clear contrast to hematological malignancies, are a consequence, rather than the cause, of malignant transformation.

This situation changed with the introduction of molecular methods to cytogenetic techniques for the analysis of chromosomal aberrations. Based on the principle of fluorescence in situ hybridization (FISH) several technologies were developed that facilitated screening of tumor genomes for cytogenetic abnormalities independent of chromosome banding, and without the need for the preparation of tumor metaphase chromosomes. Probably most relevant in the context discussed here was the development of the concept of interphase cytogenetics [12], chromosome painting [13,14], comparative genomic hybridization (CGH) in the laboratories of Pinkel, Gray, Ward and Cremer [15,16], and of spectral karyotyping (SKY) and mFISH by the Ried and Ward groups, respectively [17,18]. Interphase cytogenetics allows visualization of numerical and structural aberrations in non-dividing cells in tissue sections or cytological preparations [19], whereas chromosome painting is useful for the confirmation of suspected aberrations. CGH allows mapping of genomic imbalances in solid tumor genomes on normal reference chromosomes, or, with much higher resolution, on arrayed nucleic acid sequences (array CGH or aCGH) [20,21]. SKY or mFISH, on the other hand, greatly facilitates the interpretation of even highly complex karyotypes with unprecedented accuracy [22]. Therefore, some of the major obstacles in solid tumor cytogenetics, i.e., the difficulties associated with preparing metaphase spreads and conclusively interpreting the onslaught of observed aberrations, were overcome [10]. CGH analysis only requires the availability of genomic DNA extracted from the tumor. This, of course, also offered the opportunity to extend the methodology to the analysis of paraffin-

embedded material, and to the analysis of defined regions on histological tumor sections after microdissection [23,24]. This very feature was particularly useful for the identification of genomic imbalances that defined tumor progression from dysplastic lesions to invasive disease [25]. Molecular cytogenetic methods have been applied to the study of essentially all solid tumor entities. It is now clear that the picture of a cytogenetic chaos in carcinomas has to be revised. Several facts are now established beyond doubt:

1. carcinomas are defined by a non-random distribution of chromosomal gains and losses;
2. the distribution of these imbalances is tumor specific;
3. tumor specific chromosomal gains and losses occur before the transition to invasive disease;
4. these imbalances are not present in normal cells;
5. such imbalances often comprise entire chromosome arms or chromosomes;
6. aberrations emerging early in disease progression are usually maintained at advanced stages of the disease, in metastases, and in tumor-derived cell lines;
7. specific aneuploidies are the basis for the clonal evolution and expansion of precancerous lesions;
8. the majority of structural aberrations result in genomic copy number changes, i.e., balanced translocations, the hallmark of hematological malignancies, are rare.

In order to substantiate these statements, an in depth analysis of the landscape of chromosomal aberrations in three distinct cancer entities – cervical, colorectal (CRC), and breast cancer – will follow.

1.1. Cervical cancer

The insufficiency of conventional cytogenetic techniques for the analysis of solid tumor chromosomes as it relates to genomic imbalances is probably best reflected in cervical cancer. Despite numerous studies and attempts over decades to analyze not only invasive disease but also precursor lesions [9,26,27], only after the application of molecular cytogenetic techniques was it revealed that essentially all cervical carcinomas have an extra copy of the long arm of chromosome 3 [25,28]; this non-random distribution is shown in Fig. 1. The gain of 3q is already present in dysplastic precursors. In fact, the presence of this sole cytogenetic aberration discerns those lesions that will eventually progress from those that will not. Deduced primarily from retrospective studies of Pap smears using interphase FISH, the gain of 3q defines the point of no return in the progression to invasiveness, thereby corroborating the dominant nature of this genomic imbalance in this disease [29,30].

1.2. Colorectal cancer

CRC was more amenable to cytogenetic analyses; in fact, Bardi and colleagues, reported extensive data on CRC and derived cell lines, and plotted the results as maps of gains and losses [31,32]. The fact that alterations in chromosome 3 are not present in CRC speaks to the tumor-type specificity of these genomic changes. Instead, almost all sporadically

occurring tumors display increased copy numbers of chromosomes and chromosome arms 7, 8q, 13, and 20q, accompanied by losses of 8p, 17p, and 18 (see also Fig. 1). Similarly to 3q in cervical cancer, some of these aberrations, mainly the gain of 7 and 20q, can be already observed in preneoplastic polyps [33,34] and most, if not all, are still present in liver metastases of this disease and in cell lines derived from primary tumors or liver filiae. The plethora of CGH studies applied to map genomic imbalances in CRC convincingly confirmed these earlier results [35–41].

1.3. Breast cancer

The fact that chromosomal gains and losses define specific tumor entities also applies to breast cancer, even though the picture is a bit more complicated because of the heterogeneity of this disease [42–47]. Past studies that applied quantitative measurements of the nuclear DNA content of primary breast carcinomas unambiguously established that tumors with a higher degree of genomic instability were associated with a worse prognosis [48,49]. This allowed discernment of chiefly two groups of tumors: those that were near-diploid, with a relatively stable genome (we will describe the meaning of near-diploid below), and those that were aneuploid, displaying pronounced DNA content differences from one cell to another [50]. With higher resolution CGH techniques, these groups could be further discerned into three subtypes and it became clear that near-diploid tumors frequently carried extra copies of chromosome arm 1q and loss of 16q, also referred to “1q/16q” tumors, whereas aneuploid tumors were defined either by recurrent copy number gains on 8q and extensive chromosomal level instability, named “complex”, or by frequent focal high-level amplifications, e.g., of the oncogenes *CCND1*, *MYC*, and *ERBB2*, known as “amplifier” group [43,51,52]. More recently, this classification was further refined to normal-like, basal, luminal A and B, and her2-neu-positive sub-types, and cases described as 1q/16q based solely on the distribution of chromosomal gains and losses [53]. These categories are also reflected by specific gene expression profiles [44,47,54,55].

Cervical, colorectal, and breast cancer are just a few examples to demonstrate the perplexing conservation of genomic imbalances hidden under the complexity of the perceived cytogenetic noise (Fig. 1). This concept applies to essentially all other carcinomas as well, and can be used to distinguish one tumor entity from another. In summary, solid tumors are not governed by a cytogenetic chaos, but in fact have a surprisingly stable, tumor- and tumor stage-specific distribution of chromosomal gains and losses. The widely-held dogma that chromosomal aberrations are randomly generated as passengers during the process of tumorigenesis, rather than being drivers of the disease, was a perception disproven by advances in cytogenetic technologies over the recent past [56–58].

Of note, this non-random distribution of chromosomal gains and losses is more or less recapitulated in mouse models of epithelial tumorigenesis where the recurrence of specific genomic imbalances is observed during spontaneous transformation of normal mouse epithelial cells derived from various organs. Intriguingly, the chromosomal location and distribution of these imbalances is for the most part similar to those observed in human tumors [59]. The conservation of genomic imbalances as a dominant feature of

carcinogenesis across species boundaries arguably increases their relevance for malignant transformation.

2. Hypothesis: only one, a few, or many genes as targets of chromosomal aneuploidies?

The observations described in detail above, of course, must trigger the question as to the consequences of these so dominantly selected aneuploidies with respect to the transcriptional activity of genes on the affected chromosomes in cancer cells. There are four formal hypotheses one could put forward: (i) genomic copy number is neutral vis-á-vis the expression of resident genes; (ii) chromosomal aneuploidies target a small and limited number of genes that behave as oncogenes or tumor suppressor genes when chromosomes are gained or lost, respectively. The expression of other genes on these chromosomes is not affected and therefore does not play a role in carcinogenesis; (iii) chromosomal copy number changes affect the expression of most, if not all, genes on the altered genomic segment, however the extent to which genes other than oncogenes and tumor suppressors contribute to malignant transformation, or maintenance of the transformed state, is unclear; (iv) the biological effect of chromosomal aneuploidy is not limited to the affected chromosomal region, but also has trans effects on the transcriptional activity of genes residing in other areas of the genome. The implication of the latter two hypotheses would be a massive deregulation of the transcriptome in cancer cells with extremely complex consequences on the transcriptional equilibrium.

3. Analytical methods

The extent to which chromosomal aneuploidies impact gene expression is at the root of the enigma of tumorigenesis. It is therefore not surprising that this very question has precipitated considerable activity in the research community. Most studies aimed at unraveling the consequences of genomic copy number changes on the transcriptome integrated the mapping of chromosomal imbalances derived from CGH, or more recently aCGH, with array-based global gene expression profiles. The bioinformatic or statistic integration of genomic copy number and gene expression profiles, however, was variable, thereby making a direct comparison of independently obtained sometimes difficult. This review will therefore not focus on the different analytical methodologies, but rather on an interpretation of data.

That being said, however, we would be remiss if a brief summary of the main methodological and analytical tools that were developed and applied to interrogate the relationship between genomic copy number and resident gene expression levels is based on the following publications was not presented [47,60–70]. The calculation of false discovery rates in expression data analyses was commonly based on p-values derived from *t*-test or Wilcoxon rank-sum test, with multiple test corrections. Copy number analysis of aCGH experiments was usually based on segmentation algorithms, such as CBS (Circular Binary Segmentation) [71] and GLAD (Gain and Loss Analysis of DNA) [72]. The GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm [73] was frequently used for the statistical analysis of large sample sets. Hierarchical clustering was the most

commonly used approach for classification of samples using gene expression or copy number profiles. IPA (Ingenuity Pathway Analysis; www.ingenuity.com), DAVID (Database for Annotation, Visualization and Integrated Discovery; http://david.abcc.ncifcrf.gov/), and GSEA (Gene Set Enrichment Analysis; www.broadinstitute.org/gsea/), among others, are the statistical tools applied to calculate the significance of correlation of gene sets with different molecular pathways. The Pearson correlation or Spearman's rank correlation coefficients were used to calculate the correlation between DNA copy number changes and gene expression levels. This correlation can be established for each individual gene, or, in order to reduce inherent noise, for the average of genes on each affected chromosome, chromosome arm, or CBS-derived chromosome segments.

4. Experimental systems and results

Studies addressing the issue of genomic copy number and gene expression changes were performed utilizing primary tumors and tumor-derived cell lines. Model systems generated to specifically address this problem range from yeast to non-cancer associated aneusomies, but the majority of studies centered on aneuploidies in cancer cells. The following discussion is confined to those studies that focused more on global relationships of genomic copy number and gene expression, rather than on the consequences of localized, focal amplicons (see Table 1).

4.1. Studies in yeast and constitutional chromosomal aneuploidies in non-cancerous cells

Three studies in yeast, either based on spontaneously occurring or manipulated aneuploid mutant strains, convincingly demonstrated that genomic copy number more or less directly influences resident gene expression levels. The first study reported an effect of genomic copy number on essentially all genes residing on the aneuploid chromosomes and concluded that there is no dosage compensation for the expression level of genes affected by copy number changes [74]. Using aneuploid yeast strains, the Amon laboratory could show that genes along the entire length of the disomic chromosomes were on average more highly transcribed [75]. Recently, Pavelka and colleagues confirmed these results, and additionally demonstrated that alterations in gene expression are transmitted to the level of protein synthesis [76]. A similar picture emerged when human brain or fetal cells derived from individuals with constitutional aneuploidies affecting chromosomes 13, 18, and 21 were studied. Mao and colleagues reported a general increase in expression of genes on chromosome 21 in the cerebral cortex (and derived cell lines) from individuals with Down syndrome [77]. FitzPatrick and colleagues, who observed subtle yet significant transcriptional increases from aneuploid chromosomes that were sufficient to predict the underlying genomic imbalances, subsequently confirmed these results [78]. The Ts65Dn mouse model for human trisomy 21, in which MMU 16 is present in additional copy numbers, revealed that in addition to effects on genes residing on MMU 16, a profound effect was observed on the expression level of genes throughout the genome, consistent with the hypothesis of a global destabilization of the transcriptional equilibrium [79].

4.2. Cell lines as models

Cell lines derived from primary carcinomas or precursor lesions were widely used to measure the effect of genomic copy number on the expression of resident genes. Phillips and colleagues compared an immortalized human prostate epithelial cell line with a tumorigenic derivative (induced by co-culture with carcinoma-associated fibroblasts) and identified several specific cytogenetic changes between the two lines. Those resulted, while not uniformly, in significant changes in the expression of resident genes [80]. However, many genes with deregulated expression levels mapped to regions not affected by chromosomal copy number changes. Wolf and colleagues arrived at a similar conclusion when studying the prostate cancer cell lines PC-3, DU 145, LNCaP, and CWR22R. Again, a significant correlation of genomic copy number with gene expression levels became evident throughout the genome, both for regions with elevated and reduced copy number [81].

Similar comparisons were conducted in breast cancer cell lines: one of the first such studies used cDNA arrays for the measurement of both DNA copy number alterations and associated gene expression changes. They found that, at least for the cell line BT474, many genes were highly overexpressed in the absence of genomic amplification, and conversely, not all amplified genes were overexpressed. However, a quantitative genome wide comparison was not attempted or reported [82]. The same group, however, arrived at a different conclusion in a later study on both primary cancers and 10 derived cell lines: there they showed (i) that 62% of highly amplified genes were overexpressed, (ii) that also low level copy number changes globally influenced gene expression levels, and (iii) that at least 12% of transcriptional deregulation can be attributed to genomic imbalances. They also reported that a two-fold change in copy number caused an approximately 1.5-fold change in expression levels [44]. Neve and colleagues studied 51 breast cancer cell lines [53] by aCGH based on 2646 BAC clones and expression profiles on Affymetrix chips. By in large, the genomic aberration profiles that define subclasses of primary tumors were maintained, and so were the gene expression profiles that served as the basis for the distinction established according the data generated by Perou and Sorlie [54,55]. Most importantly in the context of this review is the finding that that gene expression levels were dependent on genomic copy number, which did not only apply to high-level genomic amplifications, but to low-level whole chromosome or chromosome arm changes as well (below, we will summarize the findings observed by the same group in primary breast cancers and compare it to those in derived cell lines). Importantly, the results justify the statement that cell lines, perhaps more than appreciated before, recapitulate not only the genomic aberration profile observed in primary cancers, but also gene expression profiles that are to a considerable extent influenced by them.

Three additional papers reported on the relationship of copy number and expression levels utilizing cell lines established from malignant gliomas, colorectal carcinomas, and the NCI-60 cell line panel: Gao and colleagues reported a study on cell lines from glioblastoma that differ from the parental line in terms of proliferative activity [83]. This change in phenotype, or in others words, this clonal evolution, is associated with a specific karyotype shift as determined by SKY. A thorough analysis of the expression levels of gained or lost chromosomes between the different clones showed a strict correlation with genomic copy

number, which ranged, in $\log_2$ values, from about 0.2 to 0.5. This dependency of transcript levels and gene copy number not only applied to entire chromosomes, but also to subchromosomal regions. A statistically significant association of genomic imbalances with gene expression levels across the cancer genome was also established by Camps and colleagues in a series of 15 colorectal cancer cell lines [84]. These interpretations were consistent with the results of Bussey and colleagues, who reported an in general positive correlation between the expression of a gene and its copy number in the NCI-60 panel of diverse cancer cell lines [85]. In a systematic study to address these relationships, Upender and colleagues developed specific model systems [60]. These authors used microcell-mediated chromosome transfer [86] to generate artificial trisomies in otherwise karyotypically normal cells. The mismatch repair-deficient colorectal cancer cell line DLD1, which is essentially diploid (the karyotype only reveals very few subtle changes) and the immortalized normal breast epithelial line hTERT-HME (46, XX) were used as recipients. Regarding DLD-1, derived cell lines carrying extra copies of human chromosomes 3, 7, and 13 were generated, and for hTERT-HME one derived line with an extra copy of chromosome 3. Cytogenetic analysis after microcell-mediated transfer confirmed the presence of trisomies for the respective chromosomes, and also proved that the experimental manipulation did not induce other changes to the karyotype. Gene expression profiling of the parental and four derived lines was conducted on cDNA arrays. The results very convincingly demonstrated a direct role of chromosomal trisomies on gene expression levels. Not all derived lines contained the same number of additional chromosomes, and some cells with just two copies persisted, but other cells now carried four chromosomes: however, the average increase in genomic copy number was 1.44 (i.e., close to trisomic), whereas the average increase in gene expression levels was 1.21. Induced changes to gene expression levels were not restricted to genes residing on the introduced chromosomes. In fact, the number of genes that were transcriptionally deregulated across the genome was higher than the one in the introduced chromosome. Whether this deregulation is random in nature, or directed, is not clear. Particularly interesting perhaps would be to identify those genes that are consistently refractory to an aneuploidy-induced deregulation: their overexpression could potentially reduce the viability of cancer cells.

4.3. Studies in primary tumors

A considerable number of publications used primary tumors to address genomic copy number and transcription level relationships. Masayesva and colleagues analyzed primary head and neck cancers and matched normal mucosa, and Xu et al. profiled 20 lymph node metastases of patients with oral squamous cell carcinoma [87,88]. Both studies, while different in focus came to the conclusion that gene expression profiles were, over large chromosomal regions, influenced by cancer specific copy number alterations. The authors of the first paper interpreted their findings such that not all genes whose expression levels were deregulated by copy number are relevant for tumorigenesis, while the latter observed in fact a dose relationship and noted that some 30% of transcripts showed a dependency with copy number. Focusing on chromosome 22 in ovarian cancers, Benetkiewicz observed a very similar circumstance [89], and so did Woo and colleagues in hepatocellular carcinomas [90]. These authors compared aCGH analyses with gene expression analyses with the goal of identifying “drivers” of tumorigenesis. Based on the projection of a dataset of gene

expression profiles from 139 primary tumors onto maps of genomic imbalances, a close dependency of expression levels with copy number changes emerged. A study by Roessler and colleagues corroborated these interpretations in the same tumor entity [91].

Acute myeloid leukemia is defined by either a normal karyotype, or by numerical aberrations of specific chromosomes, most commonly a trisomy of chromosome 8 as the sole cytogenetic abnormality. A study by Virtaneva set out to query the influence of this aberration on gene expression levels [92]. The comparison of patients with and without trisomy 8 revealed an unambiguous and direct effect of genomic copy number on resident gene expression levels. In fact, the authors observed, on average, a 30% increase in expression. A similar study by Schoch and colleagues on acute myeloid leukemia included patients, that, in addition to trisomy 8 revealed extra chromosomes 11 and 13, and losses of 7, or a deletion of the long arm of chromosome 5, essentially confirmed the results reported by Virtaneva [93]. Schoch also showed that losses affect gene expression levels slightly more strongly than gains, but in the same range (30%) as reported by Virtaneva and colleagues.

The tumor entities studied in greatest detail are breast and colorectal cancer (CRC). The results regarding cell lines derived from breast cancers were described in the respective session above. Two early papers focused mainly on high-level amplifications in primary breast cancer, and both showed an impact of localized genomic amplification on the expression of genes in these amplicons. Specifically, more than 40% of highly amplified genes were overexpressed [94,95]. The most comprehensive collection of primary breast cancers was described by Chin and colleagues, who assessed copy number changes and gene expression levels in 101 primary tumors [47]. As expected, these tumors comprised different subtypes, such as normal-like, basal-like, luminal A and luminal B, and ERBB2, as defined earlier by both expression profiling and comparative genomic hybridization [44,46,54,55,96,97]. Both, genomic aberration patterns and gene expression profiles are predictors of clinical outcome [43,44,54,55,97,98]. In the context of this review, Chin and colleagues analyzed gene expression levels of 186 genes in distinct amplicons and chromosome bands 8p11–12, 11q13–14, 17q11– 12, and 20q13, all of which are known be involved in breast cancer [47]. In congruence with the results by Hyman [95], about 30% of these targets were overexpressed. Chin and colleagues then studied the consequences of low-level copy number changes, which are common in breast cancer as well, on resident gene expression levels and could show that those genes whose expression levels were affected by low-level copy number alterations of chromosome arms or entire chromosomes belonged to the functional ontologies RNA processing, RNA metabolisms, and cellular metabolism, suggesting a supportive role for the increased metabolic requirements intrinsic to highly proliferating cancer cells.

The most extensive integration of genomic copy number and transcriptional activity was reported for colorectal cancer. The first study that was aimed at an integration of copy number and gene expression was published by Platzer and colleagues in 2002 [37]. These authors concluded that genes in regions of chromosomal copy number increase are frequently silenced. However, a closer look at the analytical procedures suggests that the thresholds for defining overexpression were probably selected too stringently. Subsequent

papers that systematically addressed this question appeared in 2006 [61,62]. The first explored some 20 rectal cancer samples and matched normal mucosa. The results showed a clear correlation on chromosomes frequently affected by copy number changes in a directionality that was expected by copy number change. In addition, the percentage of genes deregulated when comparing cancer and matched mucosa, and the percentage of those deregulated genes that were upregulated, were highest for chromosomes 13 and 20, i.e., chromosomes that are consistently gained. Interestingly, the group of mucosa samples also clearly separated into two classes, which was based on the chromosome-wide gene expression changes of some chromosomes, yet not chromosomes 13 and 20. The reasons for this separation remain unknown. Tsafrir and colleagues investigated normal colon epithelium, adenomas, and carcinomas, for a subset of which CGH analysis (either on custom arrays, retrieved from the literature, or from Affymetrix chips) and expression profiles (Affymetrix) were available. Again, the correlation of commonly affected chromosomes or chromosome arms with gene expression profiles was obvious. The authors also noted that during disease progression, copy number dependent transcriptional deregulation became more noticeable [62]. Grade and colleagues extended their observations in rectal cancer to a comprehensive dataset of primary UICC stage II and III colon tumors [63]. Concordant to the results obtained from the analysis of rectal cancers, the authors revealed a massive, aneuploidy-dependent deregulation of the colon cancer transcriptome on those chromosomes that are recurrently affected by copy number changes (chromosome arms and chromosomes 7p, 8q, 13q, and 20 as gains, and 18q as loss). Of note, precisely as in rectal cancer, the proportion of genes on chromosomes 13 and 20 was profoundly higher than on other chromosomes, attesting once more to the central role these chromosomes play in colorectal tumorigenesis. Yet another study by Habermann and colleagues came to similar conclusions [64]. Here, the authors focused on the sequential transition from normal epithelium, to adenomas, carcinomas, and metastases, in 36 patients (for eight of those samples the entire progression sequel was available). Again, the results confirmed the direct dependency of gene expression levels on chromosomal aneuploidies. Camps and colleagues extended such analyses to the entire genome: they identified, using aCGH and a Circular Binary Segmentation (CBS) algorithm, units of copy number alteration across the entire genome of colon cancer samples. This analysis revealed a highly significant ($R = 0.66709$, $P = 2.2e^{-16}$) positive correlation of segments of copy number change and gene expression levels [66]. Finally, Sheffer and colleagues convincingly demonstrated that the pattern of gene expression profiles alone is sufficient to predict genomic copy number alterations in a comprehensive dataset of colorectal carcinomas [68]. The consequences of chromosomal imbalances are visualized using different approaches and models in Figs. 2–5. In Fig. 2 we present the results of our analysis of primary colorectal carcinomas. Fig. 2A shows the global effect on the transcriptome for chromosomes that are lost, balanced, or gained, and Fig. 2B presents an individual primary colon tumor (upper panel), and the summary of gains and losses detected by aCGH in a series of 31 carcinomas. Fig. 3 presents a correlation of chromosome-wide genomic copy numbers with resident gene expression levels in colorectal carcinomas, and Fig. 4 presents such a correlation for a specific chromosome. Fig. 5 presents the correlation on a single gene level. In all instances, gene expression levels follow genomic copy number.

5. Implications and future challenges

Essentially all studies aimed at deciphering the relationship of genomic copy number and resident gene expression levels, independent of the cellular context, the species in which this question was addressed, or the degree of DNA copy number alterations, came to the conclusion that there exists a positive correlation between the two. From a teleological point of view these observations immediately trigger the following biological questions:

1. What is the relevance of aneuploidy-dependent deregulation of (global) gene expression changes with respect to the phenotype of aneuploidy-carrying cells?
2. Is one or are a few genes the drivers behind the acquisition and maintenance of aneuploidy in cancer cells? Or are most, if not all deregulated genes at the root for the continuous selection of genomic imbalances?
3. What is the relative role of the aneuploidy-dependent massive transcriptional deregulation vis-á-vis the genomic copy number based gain or loss of function of bona-fide oncogenes or tumor suppressor genes on the respective gained or lost chromosomes?
4. What are the global consequences of specific aneuploidies on the transcriptional equilibrium of cancer cells? In other words: to which extent do aneuploidies specifically (or stochastically) influence the expression of genes on other than the aneuploid chromosomes, and with that yield an effect on cellular signaling networks?
5. Why are, if there is in general a direct correlation of genomic copy number and gene expression levels, not all genes affected by this rule, and what are the cellular control mechanism involved in the escape of aneuploidy-dependent transcriptional deregulation of such genes?
6. Could an induced upregulation of such genes be utilized to reduce the proliferative fitness of aneuploid cancer cells?
7. To which extent is the early acquisition of aneuploidies in premalignant lesions, and the maintenance of them in invasive and metastatic disease, and in derived cell lines, a reflection of the tissue of origin?
8. To which extent is the higher-order nuclear structure, i.e., the nonrandom organization of chromosomes, required for aneuploidy-driven transcriptional deregulation?
9. Can we observe such an interdependency of copy number and transcriptional deregulation also in what is perceived to be the cancer stem cell?
10. And finally, what are the experimental tools that we command, or have to devise, to address these fundamental questions for a systematic and comprehensive characterization of what discerns a cancer cell from its differentiated precursor.

We believe that answering these questions will be critical to understanding one of the most important aspects of tumorigenesis, i.e., the consequences of genomic instability on the transcriptional equilibrium.

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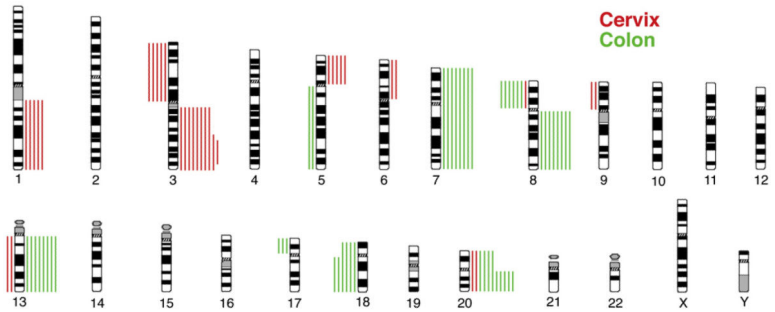

Fig. 1.

Distribution of chromosomal gains and losses in cervical and colorectal carcinomas. The distribution of genomic imbalances is tumor specific. The results are normalized to n = 10. Note that essentially all cervical carcinomas carry a gain of chromosome arm 3q, and colorectal carcinomas are defined by a recurrent gain of chromosomes 7, 8q, 13, and 20q, and losses of chromosomes 8p, 17p, and 18.

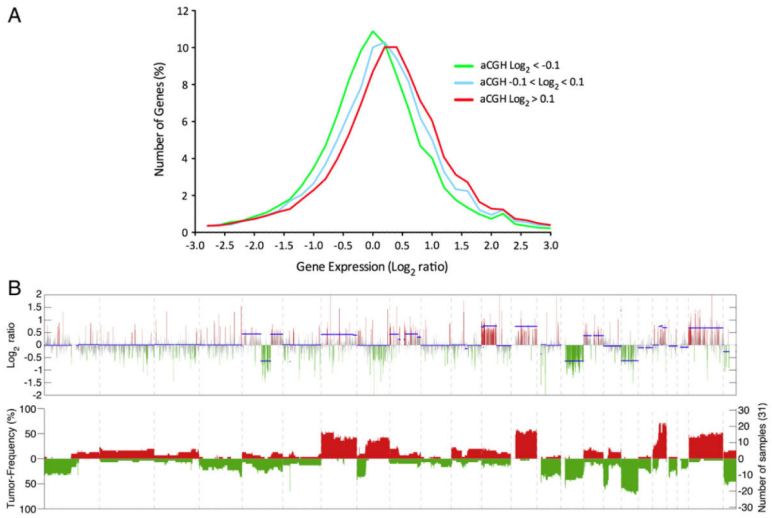

Fig. 2.

Influence of genomic copy number changes on the transcriptome. A: Shown in this graph are, in green, the distribution of gene expression levels of genes on chromosomes that are lost, in blue, the expression levels of genes on chromosomes that are balanced, and in red, the expression levels of genes on chromosomes that are present in extra copy numbers. Plotted are the percentages of genes with $\log_2$ expression values. The CGH values of genomic intervals clearly affect average gene expression levels with expression values of lost chromosomes shifted to the left, and those with extra copy numbers shifted to the right. B: The upper part of the figure shows one example of a primary colon cancer. The blue lines indicate genomic copy numbers as measured by aCGH. In general, the gain of chromosomes results in the overexpression of genes (red) and the loss of chromosomes in reduced gene expression levels (green). The lower part of the figure presents a summary plot of genomic imbalances detected in 31 primary colon cancers by aCGH. The distribution is non-random and reflected in the individual profile in the upper panel of B.

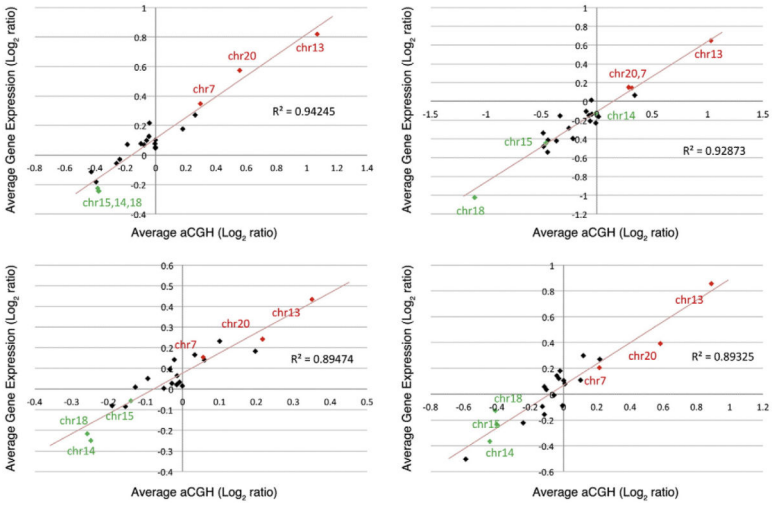

Fig. 3.

Chromosome-specific changes in gene expression levels depend on genomic copy number. This figure presents the correlation of chromosome-wide genomic copy number and gene expression levels for four individual cases of primary colorectal carcinomas. Note that certain chromosomes (i.e., 7, 13, and 20) are consistently gained, while others (14, 15, and 18) are lost. These genomic imbalances exert a direct effect on resident gene expression levels with a high coefficient of correlation.

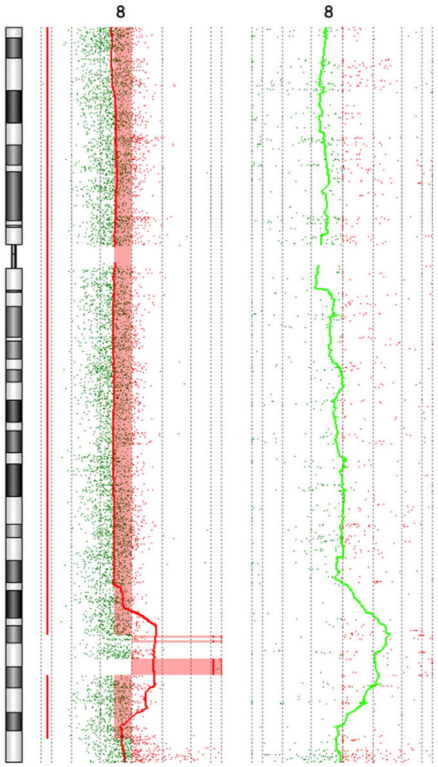

Fig. 4.

Higher-resolution display of the correlation of genomic copy number and gene expression levels for an individual chromosome. The left part of the figure shows the aCGH data for chromosome 8, and the right corresponding gene expression levels. Note that there is a direct correlation of average gene expression levels and genomic copy number.

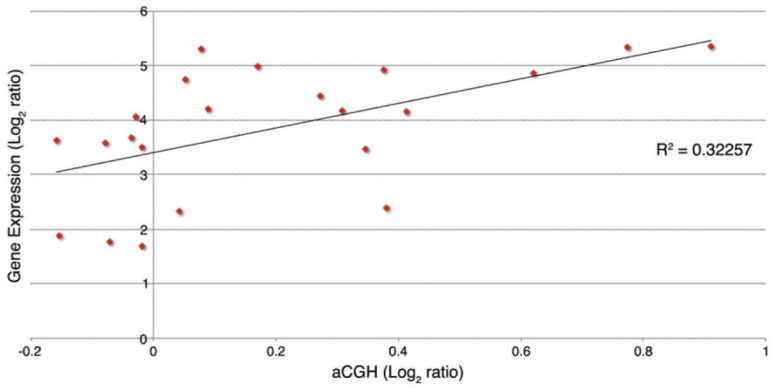

Fig. 5.

Correlation of genomic copy numbers of an individual gene (MYC) with expression levels. The interdependency of genomic copy number and expression levels is presented for the MYC oncogene. Note that expression levels correlate with genomic copy number even when genes are analyzed individually, though with a lower coefficient of correlation observed for average, chromosome-wide expression levels.

Table 1

Summary of the publications on the correlation of genomic copy number and gene expression described in this review.

Model	Tumor entity[a]	Summary	Literature
Cell lines	Prostate	Using immortalized prostate epithelial cells and the cell lines PC-3, DU145, LNCaP, CWR22R, a relative gain or loss of a chromosome, chromosomal arm or candidate target gene usually resulted in a statistically significant increase or decrease, respectively, in the average expression level of a proportion of genes on the chromosome.	[80,81]
	Breast	These studies showed that highly amplified genes were also highly expressed in a genome-wide approach, that both high- and low-level copy number changes had a substantial impact on gene expression, with 44% of the highly amplified genes showing overexpression. These interpretations were supported in an extensive set of 51 breast cell lines comprising different breast cancer subtypes.	[44,53,82,95]
	Glioblastoma	Integration of SKY, aCGH and gene expression analysis in four cell lines, DB-P, DB-A2, DB-A6, and A2-BH7, cells showed that fold increase or decrease in the chromosome content ratio is virtually the same as the transcriptome ratio.	[83]
	Colorectal	Analysis of 15 colorectal cancer cell lines, including mismatch repair proficient and deficient lines, showed positive genome-wide correlation between copy number determined by aCGH and corresponding average gene expression.	[84]
	NCI-60 panel	The authors used the NCI-60 panel to study the relationships between DNA copy number, mRNA expression level, and drug sensitivity. They concluded that genomic copy number is one factor that can influence gene expression levels.	[85]
	Aneuploidy models	Introduction of an extra chromosome in a diploid colorectal cancer cell line or in an immortalized HME line resulted in average increase of gene expression for the genes on the introduced chromosome, and an additional deregulation of genes on other chromosomes.	[60]
Primary tumors	Head and neck	Loss and gain of chromosomal material can alter gene expression over large chromosomal regions. Loss of chromosome arm 3p, established as a genetic alteration in head and neck cancer, resulted in consistent underexpression in all tumors and significantly so in selected tumors with documented 3p allelic imbalances.	[87]
	Oral	Isolated cells from lymph nodes of 20 patients with oral squamous cell carcinoma were analyzed. Genome region 11q13.2–13.3 showed the highest correlation between DNA copy number alterations and gene expression, leading the authors to conclude that genes exhibiting high levels of correlation may have biological impact on carcino-genesis and cancer progression.	[88]
	Ovarian	The authors identified three distinct tumor groups based on the copy number profile, including 33 regions of losses and gains for which mRNA levels were low and high, respectively. Several of the deregulated genes play roles in cell cycle control and the induction of apoptosis.	[89]
	Liver	Transcriptome correlation maps for 139 hepatocellular carcinomas identified 48 regions in which gene expression levels were likely to be copy number-dependent. Somatic copy number alterations correlated with expression of 27.3% of genes analyzed in a cohort of 76 hepatocellular carcinoma samples from patients with hepatitis B virus infection.	[90,91]
	Acute myeloid leukemia (AML)	In hematological neoplasias, in which specific aneuploidies occur in a more discrete fashion, trisomy 8, 11, or 13 resulted in increased gene expression levels, while in AML with monosomy 7 and deletion 5q the median expression was lower.	[92,93]

Model	Tumor entity[a]	Summary	Literature
	Breast	By analyzing copy number alterations, breast tumors form three distinct subtypes known as the “1q/16q” (or “simple”), “complex,” and “amplifier”. Genome-aberration-driven deregulation of gene expression affected about 10% of the genome interrogated when studying a set of 145 primary breast tumors.	[44,47,82,94,95]
	Colorectal	In colorectal carcinomas, tumorigenesis requires specific copy number alterations, e.g., gains of chromosomes 7, 8q, 13, and 20q, and losses of 8p, 17p, and 18. These aneuploidies influence gene expression levels in adenomas, carcinomas, and metastases.	[61–64,66,68]

[a]Order according to their appearance in the text.