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CGH microarrays and cancer

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Genetic alterations are a key feature of cancer cells and typically target biological processes and pathways that contribute to cancer pathogenesis. Array-based comparative genomic hybridization (aCGH) has provided a wealth of new information on copy number changes in cancer on a genome-wide level and aCGH data have also been utilized in cancer classification. More importantly, aCGH analyses have allowed highly accurate localization of specific genetic alterations that, for example, are associated with tumor progression, therapy response, or patient outcome. The genes involved in these aberrations are likely to contribute to cancer pathogenesis, and the high-resolution mapping by aCGH greatly facilitates the subsequent identification of these cancer-associated genes.

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Introduction

DNA copy number changes are common in cancer and lead to altered expression and function of genes residing within the affected region of the genome. Traditionally, such segments in the tumor genome are thought to harbor either oncogenes or tumor suppressor genes depending on whether they are present in increased or decreased copy number, respectively. Identification of regions with copy number aberrations and especially the genes involved thus offers a basis for better understanding of cancer development and more importantly is likely to provide improved tools for clinical management of cancer, such as new diagnostics and therapeutic targets.

Comparative genomic hybridization (CGH) technique was developed in the early 1990s for genome-wide characterization of copy number changes, especially in cancer [1]. In this technique, total genomic DNA is isolated from tumor and normal control cells, labeled

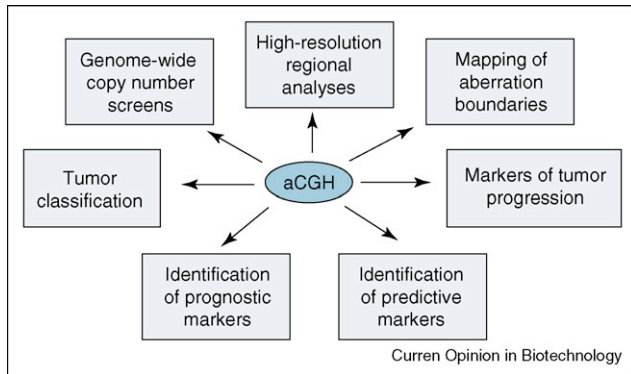
with different fluorochromes and hybridized to normal metaphase chromosomes. Differences in the tumor to normal fluorescence ratio along the metaphase chromosomes are then quantitated and reflect changes in the DNA sequence copy number in the tumor genome. Subsequently array-based CGH (aCGH), where arrays of genomic sequences replaced the metaphase chromosomes as hybridization targets, was established [2–4] and solved many of the technical difficulties and problems caused by working with cytogenetic chromosome preparations. The main advantage of aCGH is, however, the ability to perform copy number analyses with much higher resolution than was ever possible using chromosomal CGH [5–7].

Different kinds of aCGH platforms are currently available ranging from arrayed bacterial artificial chromosome (BAC) clones to cDNA clones and various oligonucleotide-based formats [5–7]. The technical and methodological issues of aCGH as well as the general applications of this technology both in cancer research and in human genetics have been recently discussed in several excellent reviews [5–7]. This article will concentrate on recent discoveries obtained in cancer research through the aCGH approach and will especially focus on studies published within a two-year period from 2005 to 2007. The use of aCGH for genome-wide screening of copy number changes as well as for targeted analyses of specific regions of interest will be discussed. Studies with more focused applications, such as tumor classification or identification of specific copy number changes associated with clinicopathological tumor characteristics, tumor progression, patient outcome, and therapy response, will also be covered (Figure 1).

Global analysis of copy number aberrations and identification of putative target genes

A vast number of tumor samples representing both common tumor types, such as breast [8–11] and colorectal cancers [12,13], as well as more rare tumor entities, including gastrointestinal stromal tumors [14–16], insulinomas [17], and ependymomas [18], have been analyzed in genome-wide aCGH studies. These studies have sought to provide a comprehensive high-resolution view of copy number changes in various tumor types and have provided a wealth of new information on the patterns of copy number alterations occurring during cancer development. A number of regions that are frequently involved in gains and losses have been identified. For example, analysis of ependymomas [18] disclosed multiple frequently occurring regions of both gain (2q23, 7p21, 12p, 13q21.1, and 20p12) and loss (5q31, 6q26, 7q36,

Figure 1



Applications of aCGH in cancer research.

15q21.1, 16q24, 17p13.3, 19p13.2, and 22q13.3) thus illustrating the extent of copy number aberrations in cancer genomes.

In addition to genome-wide surveys, targeted analyses utilizing specifically constructed arrays aiming at providing complete tiling path coverage of a specific region of the genome have become increasingly feasible [19,20–23]. Such studies have been applied for the characterization of common copy number changes at the q-arm of chromosome 8 in breast cancer where an extremely complex pattern of copy number changes with alternating areas of amplification and deletion was revealed [23]. More specifically, this study identified a total of six distinct amplicons together with three deletions at the 8q21 and 8q24 regions in breast cancer [23]. Similarly, analysis of meningiomas using a chromosome 1 specific tiling path array disclosed four separate commonly deleted candidate loci [19]. The above-mentioned examples demonstrate that copy number changes previously thought to represent simple gains or losses can indeed consist of complex discontinuous sets of aberrations that can only be discovered with the use of the high-resolution technologies.

The ultimate goal of all aCGH studies is to pinpoint the locations of cancer-associated genes as accurately as possible. The application of increased resolution array platforms is thus expected to facilitate the subsequent identification of target genes. Especially the use of oligonucleotide arrays has further increased the mapping resolution of aCGH and allows extremely precise definition of aberration boundaries and breakpoints, with a theoretical resolution of less than a kilobase [24,25]. One of the clear advantages of the increased mapping resolution of aCGH has been the more straightforward identification of small homozygous deletions, pinpointing the possible locations of tumor suppressor genes [26–28,29,30,31]. For example, analysis of mantle cell lymphomas identified several

regions of homozygous deletions, one of which (2q13) was subsequently shown to target the proapoptotic *BIM* gene [27]. A similar screen in ovarian cancers disclosed a total of 27 homozygous deletions including one corresponding to the well-known *RBI* tumor suppressor gene [30]. Likewise, analysis of oral cancers revealed a homozygous deletion of the *FAT* gene, a member of the human cadherin superfamily [31]. These examples illustrate that high-resolution aCGH has indeed improved the detection of homozygous deletions in cancer and thereby facilitated the identification putative novel tumor suppressor genes.

The increased resolution of aCGH has also recently led to the discovery that translocations, that by cytogenetic analysis have been considered to be balanced, frequently involve copy number gains or losses [32,33]. For instance, analysis of cytogenetically well-characterized prostate cancer cell lines revealed that a high fraction (80–92%) of translocations was accompanied by copy number changes [33]. It remains to be seen whether these translocation-associated copy number aberrations actually lead to relevant changes in cancer cell function or whether they simply reflect mechanistic events related to the breaking and fusion of the chromosomes.

Tumor classification by aCGH

Previous genome-wide copy number analyses have indicated that different tumor types typically possess more or less specific sets of genetic changes although some individual aberrations, such as amplification of the *ERBB2* locus at 17q12, can indeed be observed across multiple tumor types. To specifically explore the utility of copy number patterns for tumor classification, Jong *et al.* performed a meta-analysis combining aCGH data from 373 primary tumors obtained using three different array platforms (BAC, cDNA, and oligo) in four different institutes [34]. Importantly, no platform or institute specific patterns were highlighted suggesting that copy number data derived from different laboratories using different array formats can indeed be easily merged. Clustering analysis revealed that tumors were separated not only according to their tissue of origin (e.g. lymphomas, breast, colon, and prostate cancers were found in their own clusters) but also according to their embryonic origin (hematopoietic, mesenchymal, and epithelial tissues) [34]. This meta-analysis thus confirms that tumor type specific copy number patterns do exist and can be used for efficient tumor classification.

Genomic copy number profiles can also distinguish distinct subgroups within histologically defined disease entities [10,35–37]. In multiple myeloma, unsupervised clustering of aCGH data was able to divide cancer cases into specific subgroups that also showed differences in clinical outcomes [36]. Similar subclassification of tumors has also been reported in other tumor types. In breast cancer, copy number patterns classified tumors into

specific subtypes that correspond to those previously discovered through expression profiling [10]. Detailed characterization of copy number changes can thus improve disease classification and may identify clinically useful subgroups of patients.

Clinical significance of copy number changes in cancer

Association of specific genetic changes or patterns of changes to known tumor characteristics, tumor progression, or patient outcome has been one area of interest in aCGH studies. As might be expected, comparison of tumor samples representing different stages of tumor development, such as premalignant or *in situ* lesions, invasive cancers, and metastatic disease, has demonstrated that the overall number of copy number changes increases during tumor progression [10,17,38–42]. For example, in Wilms's tumors increased number of aberrations was associated with tumor progression and relapse [42]. In addition, a specific genetic change, the loss of 17p, was also linked to tumor progression [42] indicating that this aberration is probably a late event in tumor development. Specific genetic aberrations have also been linked with certain clinicopathological tumor features. As an example, gain of 1q and loss of 5q were observed more frequently in estrogen receptor negative breast tumors [10]. Such associations further imply that specific genetic events are involved in the development of different clinically relevant subgroups of tumors.

In addition to tumor progression, the high number of copy number aberrations has also been linked with poor patient prognosis [37,38], and the identification of specific genetic aberrations associated with patient outcome has been the goal in a number of aCGH studies [10,13,36,37,43–46,47•,48]. Several different aberrations have been implicated in different tumor types and both increased and decreased copy number are found to have prognostic significance. For instance, 17q23.2-qter gains and 17p13.1–p13.3 losses were indicative of poor patient outcome in medulloblastomas [44], whereas gain of 1q or loss of chromosome 13 were associated with poor prognosis in multiple myeloma [36]. In ovarian cancer, amplification at 5q31–q35 was linked with poor prognosis, whereas losses at 4p16 seemed to provide a favorable outcome [47•]. Moreover, losses on 1p36 and 21q22 were shown to represent independent indicators of poor prognosis in colorectal cancer [13], whereas amplification of 20q11.1 had similar independent prognostic value in giant cell tumors of the bone [45]. Interestingly, some aberrations, such as losses of 1p in mantle cell lymphomas and diffused large B cell lymphomas, have also been linked with good prognosis [37,43]. These data have obvious clinical significance and identification of target genes for such outcome-associated regions, especially those linked with poor prognosis, is likely to provide new information on cancer progression.

Finally, specific genetic aberrations discovered by aCGH have also been linked to differential response to various cancer therapies [49,50,51•]. Increased number of genetic changes was shown to be associated with therapy resistance in ovarian cancer where twice as many aberrations were observed in the treatment resistant tumor group as compared with the sensitive tumor group [49]. Furthermore, data from this study also showed that losses of 1p36.33 and gains of 17q11.2 were found more often in tumors that were resistant to treatment, whereas losses of 13q12–q13 were seen in sensitive cases [49]. Another study on ovarian cancer highlighted multiple different genetic aberrations associated with chemoresistance and suggested that losses of 13q32.1 and 8p21.1 were the most reliable markers of treatment resistant disease [51•]. Contrary to ovarian cancers, analysis of breast tumor samples from patients with or without a recurrence after tamoxifen treatment did not reveal any difference in the overall frequency of copy number changes between the two tumor groups [50]. However, a specific set of aberrations, namely losses of 11p15.5–p15.4, 1p36.33, 11q13.1, and 11p11.2, were found significantly more often in the recurrence group thus indicating that these loci might harbor genes associated with treatment resistance and tumor progression. Since treatment resistance is such an important clinical problem, similar studies on other tumor types are clearly warranted.

Conclusions

During the past two years, a large number of studies utilizing the aCGH technology in cancer have been published. These studies highlight the overall patterns of copy number aberrations in various tumor types and identify in high-resolution-specific genetic alterations associated with certain tumor entities, disease progression, therapy response, or patient outcome. Thereby aCGH data provide an excellent starting point for the identification of genes involved in these aberrations. However, it has to be kept in mind that aCGH merely points to the region of interest, and functional analyses are always necessary to establish the actual contribution of putative target genes to disease pathogenesis. The data derived from aCGH studies present an essential contribution to our knowledge on cancer-associated genetic aberrations and illustrate that aCGH technology still continues to function as an important tool in cancer research.

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