The Application of Single Nucleotide Polymorphism Microarrays in Cancer Research

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Abstract: The development of microarray technology has had a significant impact on the genetic analysis of human disease. The recently developed single nucleotide polymorphism (SNP**)** array can be used to measure both DNA polymorphism and dosage changes. Our laboratory has applied SNP microarray analysis to uncover frequent uniparental disomies and sub-microscopic genomic copy number gains and losses in different cancers. This review will focus on the wide range of applications of SNP microarray analysis to cancer research. SNP array genotyping can determine loss of heterozygosity, genomic copy number changes and DNA methylation alterations of cancer cells. The same technology can also be used to investigate allelic association in cancers. Therefore, it can be applied to the identification of cancer predisposition genes, oncogenes and tumor suppressor genes in specific types of tumors. As a consequence, they have potential in cancer risk assessment, diagnosis, prognosis and treatment selection.

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INTRODUCTION

Cancer development is accompanied by multiple genetic alterations including chromosomal copy number and structural changes. Identification of all genetic alterations is essential for a full understanding of the etiology of human cancer. Genetic analysis using a genome-wide detection tool is an essential approach to uncover all abnormalities and is also an efficient way to identify key genetic events, such as activation of oncogenes and inactivation of tumor suppressor genes in cancer development and progression. Such an approach can lead to quick discovery of genetic markers for cancer risk assessment, diagnosis and prognosis. Eventually, the full mapping of genotype and genetic alterations may be used for individually stratified medicine.

Karyotyping, the cytogenetic method analyzing the global genetic alterations at the chromosomal level has played an important role in our understanding of human cancers since the 1970's. Karyotype analysis has led to the identification of tumorigenic fusion genes and tumor suppressor genes and is currently used clinically for the diagnosis and prognosis of haematological malignancies. However, the general application of karyotyping is limited, since it is based on analyzing metaphase cells which are difficult to obtain in many solid tumors. Furthermore the position of chromosome breakpoints needs to be defined by fluorescence in situ hybridization (FISH) analysis.

The development of comparative genomic hybridization (CGH) in 1992 has transformed the genome wide analysis of cancer genetic alterations [1]. CGH can detect DNA copy number changes across the entire genome of a tumor sample in a single experiment by comparing the hybridization signal intensity of a tumor sample against a reference sample along the chromosomes. The initial development of CGH was applied on metaphase chromosomes [1]. Although it has already made a significant impact on cancer research and has been used to identify frequent chromosome region copy number gains and losses in many tumors, particularly solid cancers, the resolution of conventional CGH is low. Changes smaller than 2Mb are undetectable by chromosomal CGH. In 1998, Pinkel *et al.* [2] applied array technology for high-resolution CGH analysis. In array-CGH, the metaphase chromosomes are replaced by cloned DNA fragments with known genomic locations. The cloned DNA fragments can be genomic DNA or cDNA which are spotted on a glass slide or other supporting materials. As in chromosomal CGH, the DNA copy number changes are shown by the fluorescence ratio between the test and control samples. With array-CGH, sub-microscopic aberrations can be detected.

Tumor suppressor gene plays an important role in tumorigenesis, and can be detected by deletion and mutation analyses. They can also frequently be indicated by loss-of-heterozygosity (LOH) which can be picked up by genotyping analysis. The development of single nucleotide polymorphism (SNP) arrays [3] enables simultaneous detection of a large number of DNA polymorphic loci in a simple way. Further technical developments make SNP arrays capable of analyzing both signal intensity variations and changes in allelic composition in parallel [4, 5]. SNP arrays can also detect both copy number changes and copy-neutral LOH events [6-8]. Fan *et al.* have provided a detailed review of the mechanism of different SNP genotyping methods [9]. In this review, we will focus on the stages of SNP array development and its applications in cancer research.

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THE DEVELOPMENT OF SNP MICROARRAY TECHNOLOGY

SNP array technology was developed in 1998 for genotyping [3]. Since then, the technique has been improved dramatically and has become one of the most powerful genomic analysis tools. The different development stages of SNP array technology are listed in Table **1**.

The first SNP array contained 558 loci, and SNPs present within a sample amplified by multiplex polymerase chain reaction (PCR), in which primer pairs from many different loci were combined in a single reaction [3]. Amplified DNA was then hybridized on the SNP array to detect the genotyping of the 558 SNPs in the sample. A large amount of primers were required to amplify these multiple SNPs in a sample in the multiplex PCR approach for array analysis. However, primer dimer formation limited the number of primer pairs that could be included in a single PCR reaction. Therefore, the sample preparation using this protocol was still labour-extensive for high density SNP array analysis. Therefore, it took two years for this technique to be applied to cancer research. In 2000, two groups separately applied SNP array analysis for multiple detection of LOH and allelic imbalance (AI) in human cancers [10, 11]. The microarray company, Affymetrix, has improved the confidence of SNP array genotyping by interrogating on the array additional offset probes for each SNP locus [11]. The low resolution SNP array has been applied to human tumor samples mainly for LOH and allele imbalance analysis [10, 11]. Several approaches have been used to improve the capacity of multiplex PCR [9]. Among them, the GoldenGate assay [12] is one of the successful highly multiplexed PCR-based SNP genotyping method which the company Illumina has adopted for their commercial SNP array chips. Although these modifications improved the number of SNPs that can be analyzed, the multiplex PCR approach still limits the member of SNPs that can be analyzed.

In 2003, researchers at Affymetrix developed the whole-genome sampling method for SNP genotyping [13, 14]. This approach amplified genomic DNA pre-cut by a restriction enzyme. After digestion of the genomic DNA and ligation of primers, the amplification step was specifically designed to amplify DNA fragments between 400 to 800 base pairs. Using this approach, thousands of SNPs could be analyzed simultaneously. Using XbaI digestion, commercial SNP arrays containing 10,000 SNPs and accompanied by a sample preparation kit were produced. This development made the spot density and the genomic resolution of SNP array analysis higher than that of the 1Mb bacterial artificial chromosome array and cDNA arrays used commonly at that time. Since then, the SNP array has been rapidly applied to many human tumors. The application of whole-genome DNA amplification techniques in combination with SNP array genotyping [15-17] and genomic copy number analysis have been evaluated [4]. The combined genotyping and genomic copy number analysis has made the SNP array a unique technique in cancer genomic research, and revealed many new genetic features in cancer cells such as acquired uniparental disomy (UPD) [7, 8]. In the last couple of years, Affymetrix has improved the coverage of their SNP array chips further into 100K and then 500K by selecting different enzymes to fragment genomic DNA. The 500K SNP array achieved a genomic resolution of average 5Kb per SNP. Combining the 500K SNP array with 24-colour FISH analyses, we developed a high resolution karyotyping approach which can define the majority of chromosome rearrangement breakpoints to 5-50 Kb [18].

However, the Affymetrix enzyme digestion and the single primer amplification approach still has its limitations. While the selection of enzyme digested DNA fragments reduces the genomic complexity, the selected DNA only represents SNPs in a proportion of the human genome. The PCR amplification of digested DNA also limited the application of these SNP arrays in analyzing degraded DNA. The majority of human clinical cancer tissues are stored as formalin-fixed paraffin-embedded (FFPE) blocks which are invaluable for retrospective studies. Although in certain FFPE blocks, DNA is well preserved so that the fragments are large enough for whole-genome sampling analysis, DNA from the majority of tissues kept as FFPE materials is degraded. Many of the DNA fragments are not long enough to be cut twice by the enzyme, and are therefore subsequently excluded from PCR amplification. Although the application of Affymetrix 10K array analysis on FFPE samples has been reported [19], in our experience, only less than 10% of FFPE samples can be successfully analyzed by the Affymetrix 10K or 100K

Table 1. Historical Stages of the SNP Array Technology and Application Development

Years	Events
1998	First test of SNP array analysis (558 SNPs)
2000	First application of SNP array analysis on cancer
2003	Affymetrix developed 10K SNP array using single primer amplification of restriction enzyme digested genomic DNA
2004	SNP array validated for DNA copy number analysis
2004	SNP array application whole genome amplified DNA samples
2005	Development of whole-genome genotyping approach which should be scalable for simultaneous analysis of all SNPs in the genome.
2006	Development of the high resolution karyotyping approach by combined SNP and 24-color FISH analyses

SNP arrays. In this respect, samples prepared without restriction enzyme digestion worked better for SNP array analysis [20, 21].

Recently, another high throughput genotyping method was been developed by hybridizing the whole genomic DNA to an array of locus-specific capture probes [22, 23]. The SNP genotypes are scored using enzymatic allelic discrimination. Although the SNP array chips currently available for this type of SNP genotyping are limited to 550,000 loci, this principle of SNP array analysis makes the number of SNPs in one chip scalable. Using this approach, theoretically all SNPs existing in the genome can be analyzed in one experiment, and in principle it is applicable to degraded DNA such as that extracted from FFPE materials. Illumina has developed this approach, making BeadChips for high density SNP array analysis.

Accompanying the technical development of microarray arrays, many SNP array analysis tools have been developed. Lin *et al.* [24] have reported software that can pool replicates to make LOH calls and visualize SNP and LOH data along chromosomes. Using this software, statistical inference to identify shared LOH regions and sample clustering analysis is available. With the validation of high density SNP array for DNA copy number analysis, both robust algorithms and statistical methods to detect gained and/or lost chromosome regions using SNP array hybridization data were also designed [25, 26]. Laframboise *et al.* further developed software for probe-level allele-specific quantitation (PLASQ) [27, 28]. This procedure analyzes allelic specific copy number changes which can determine not just the region but also the haplotype of amplifications and deletions. Using this software, they found that amplification in lung cancer is essentially monoallelic either due to germ line or somatic variation [28]. Affymetrix has also produced similar SNP array data analyzing software for allelic specific DNA copy number changes and allelic genotyping called Copy Number Analysis With Regression and Tree (CARAT) [29]. A web-accessible SNP array analysis tool, SNPscan, was established for multiple types, combined or separated genotyping and genomic dosage analyses [30]. In our laboratory, we have developed software, GOLF, for parallel detailed analysis of LOH and DNA copy number changes along the chromosomes. Using this software, gene expression data from Affymetrix gene expression or exon arrays can also be analyzed along the chromosomes in parallel.

APPLICATION OF SNP ARRAY IN CANCER RESEARCH

Due to the complexity of genetic alterations in cancer cells, high density SNP array analysis is a very demanding technique in the field of cancer research. Here we describe the main areas of its application, and the tumor types analyzed by SNP array technology are summarized in Table **2**.

AI and LOH Analysis

The initial purpose of SNP array development was to genotype multiple SNPs simultaneously. Therefore they were first used in cancer research for LOH and AI analyses which are important for tumor suppressor gene identification. Before the development of the single primer whole-genome sampling analysis approach and the release of the Affymetrix 10K arrays, SNP array analysis was only used to study AI in cancer cells. In 2000, Lindblad-Toh *et al.* first applied SNPs array in a LOH study of human cancers. Analyzing small-cell lung cancer and control DNA samples, they found that the SNP arrays detected the same patterns of LOH as simple sequence length polymorphism (SSLPs or microsatellites) analysis [10]. Subsequently, Primdahl *et al.* applied SNP arrays on bladder cancer and found that AI occurred more frequently in T2-4 than T1 tumors. A new AI area was found on 6p [31]. Using the same approach, a LOH region on 6p was also found in prostate cancer [32]. Hoque *et al.*, investigating bladder cancer, proved that SNP array analysis picked up LOH patterns consistent with those detected from microsatellite allelotype analysis, but with additional information [33]. They applied this technique further in bladder cancer detection by analyzing urine sediment [34]. In 2004, Janne *et al.* demonstrated that small-cell lung cancer could be separated from non-small-cell lung cancer by LOH analysis using the 1500 low density SNP arrays and they further validated the efficiency and reliability of the 10K array for LOH analysis [35]. In breast cancer, SNP array analysis detected LOH patterns associated with tumor subgroups which were also partially defined by gene expres-

Table 2. Tumor Types have been Analyzed by SNP Array

Analyses	Tumor types
LOH	bladder cancer (4), lung cancer (2), prostate cancer (2), breast cancer (2), colorectal cancer (2), oral squamous cell carcinoma (2), tongue squamous cell carcinoma (1), renal cancer (1), acute lymphoblastic leukemia (1), pheochromocytoma (1), phyllodes tumor (1), fibroadenoma of the breast (1), osteosarcoma (1), neuroblastoma (1)
CGH	lung cancer (4), prostate cancer (3), colorectal cancer (1), ovarian cancer (1), melanoma (1)
Combined approach	acute myeloid leukemia (3), breast cancer (1), colorectal cancer (1), ovarian cancer (1), pancreatic cancer (1), hepatocellular carcinoma (1), oral cancer (1), basal cell carcinoma (1), myeloma (1), lymphoma (1), testicular germ cell tumor (1)
Linkage	colorectal cancer (2), hereditary mixed polyposis (colorectal cancer) (1), prostate cancer (1), chronic lymphocytic leukemia (1), esophageal cancer (1), pituitary adenoma (1)
DNA methylation	leukemia (1), Wilm's tumor (1), lung cancer (1)

Notes: The numbers in parentheses show the numbers of reports in the literature.

sion patterns [36]. Huang *et al.* [37] demonstrated that using the contiguous stretches of homozygous markers, LOH can be detected in tumor samples without analyzing the matched normal control samples. This SNP array LOH analysis using unpaired tumor samples was further validated by other research teams [38, 39]. Smoker and non-smoker oral cavity squamous cell carcinomas have been analyzed for AI using SNP array, but no difference was found [40]. In 2005, Koed *et al.* demonstrated the clinical potential using 10K SNP array for AI analysis in bladder cancer. In agreement with the results from Primdahl and colleagues, they found that AI was strongly stage-dependent. In addition, they found that cancers from different locations were associated with different frequencies of allelic imbalance. Seven out of eight tumors involving the upper urinary tract were genomically stable [41]. Irving *et al.* demonstrated that high density SNP arrays could detect LOH associated with relapse and bad prognosis in childhood lymphoblastic leukaemia [42]. Dahia *et al.*, using SNP array analysis, identified high frequency of LOH in the pheochromocytoma susceptibility loci. This analysis also further defined the locus to <2 cM [43]. Gaasenbeek *et al.* combined array-CGH with SNP array analyses to demonstrate the multiple forms of chromosomal instability in colorectal cancers [44]. Wang *et al.* applied the SNP array for LOH analysis in phyllodes tumor and fibroadenoma of breast. LOH was frequently found in phyllodes tumors and primary tumors shared common regions of LOH with paired recurrences from the same patient. However, LOH was rare in fibroademomas [45]. LOH analysis using SNP arrays was also conducted in renal cell cancer [46].

Clearly, SNP array analysis, detecting multiple polymorphism loci in one experiment, even at low density has advantages over the traditional SSLP analysis. Comparison studies of SSLP and SNP array analysis showed that SNP array results generally agree with the SSLP analysis and the accuracy of SNP array analysis is even higher than SSLP [10]. With the development of high density SNP arrays, the advantage of SNP array analysis over the traditional SSLP technique for LOH analysis is more significant. Hundreds of thousands of loci can be detected simultaneously for AI, making the genome-wide screen of tumor suppressor genes possible.

DNA Copy Number Aberration Analysis

With the development of the high-density SNP arrays, genomic resolution higher than any other array platforms has been achieved. Due to the high resolution of SNP arrays, their use in DNA copy number analysis had been explored soon after Affymetrix released its 10K array analysis system [4, 47]. Since then, SNP arrays have been applied to many tumor types for genomic copy number changes (Table **2**). Rubin *et al.* used SNP array analysis to detect the copy number gain of the 8q region, where the prostate cancer overexpressed androgen regulated gene, *TPD52* is located [48]. Zhao *et al.* detected recurrent homozygous deletion and chromosome amplification in lung cancer using the 10K array [49]. Analyzing a large number of lung cancer cell lines using SNP array technology, Sato *et al.* detected more homozygous deletions and confirmed the most frequent deletion regions on 9p using PCR analysis of an even larger series of cell lines [50]. Garraway *et al.*, combining high density SNP array analysis with gene expression signatures, identified a novel melanoma amplicon and its target gene, *MITF*. *MITF* was associated with metastatic disease and was negatively correlated with patient survival [51]. Koochekpour *et al.* [52], used SNP arrays to detect amplification of the *PSAP* gene which played a role in prostate carcinogenesis. Park *et al.* used SNP array analysis to identify, in ovarian cancer, an amplicon at 19p13.12, and further gene expression analysis identified *Notch3* as the target gene [53]. Recently, the possibility of using SNP array to generate DNA copy number changes for tumor classification has been evaluated [54]. The SNP array has also been used to define the deleted region on 9p in mesothelioma cell lines [55] and chromosomal alterations associated with cancer cell platinum resistance [56]. In pancreatic cancer, many new homozygous deletions have been detected [39]. The application of 100K SNP array in prostate cancer genetic analysis revealed small size novel alterations [57].

The currently available 500K SNP array has an average coverage of 5Kb per SNP. We have applied the 500K SNP array in leukemia and prostate cancer studies and detected many sub-microscopic deletions and amplifications that were not previously identified using lower resolution methods (Fig. **1**). Many of these sub-microscopic deletions are homo-

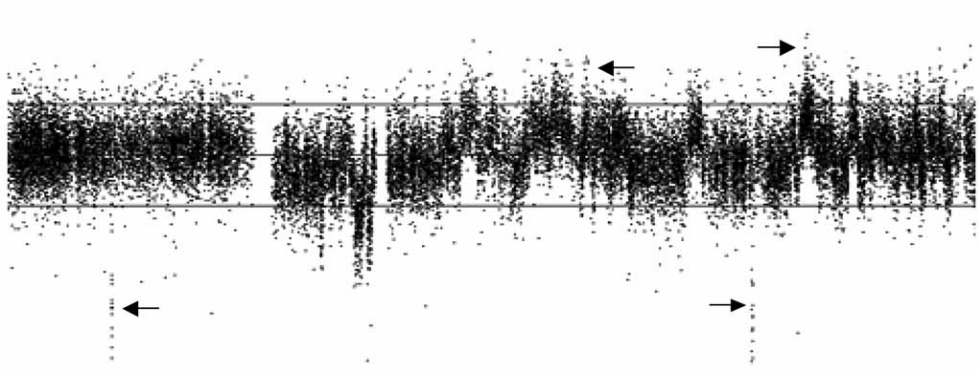

Fig. (1). An example of complex chromosome copy number alterations detected by 500K SNP array analysis. This image shows multiple small gains and losses on chromosome 5 including sub-microscopic chromosomal amplifications and deletions (arrows) around 100 Kb in the PC3 prostate cancer cell line. The middle horizontal grey line represents log2 ratio of 0 compared to normal controls; the bottom and upper lines represent log2 ratios of –1 and 1.

zygous. The finding of sub-microscopic deletions in cytogenetically normal karyotyped leukemia cells is invaluable in understanding the aetiology of this malignancy.

Combined LOH and DNA Copy Number Analysis

As SNP array analysis generates information on both LOH and DNA copy number changes, it provides a unique platform for combined genotyping and DNA copy number alteration analysis. Actually, this combined analysis is the most rewarding area of SNP array application in cancer research. Soon after the development of the 10K SNP arrays, in 2004, four groups separately validated the feasibility of using SNP data for both genotyping and DNA copy number analysis [4, 5, 15, 37]. Whole genome amplified DNA can also be used reliably for both type of analyses [5], and Huang *et al.* [37] demonstrated that both LOH and DNA copy number analyses could tolerate certain levels of normal contamination in tumor samples. In cancer, LOH may not always accompany DNA copy number loss, either through mitotic recombination, chromosome non-disjunction and loss of one parental chromosome and gain of opposite parental material. Analyzing both the LOH and DNA copy number changes, our laboratory pioneered the application of SNP analysis in the study of LOH without loss of DNA copy number in human malignancies [6-8]. In acute myeloid leukemia, we found a high frequency (20%) of UPD, and UPD regions on chromosome 19 coincided with a previously identified homozygous mutation in the *CEBPA* gene [7]. Further mutation screening identified mutations at four distinct loci, *WT1, FLT3, CEBPA*, and *RUNX1*, within the UPD regions in 7 out of 13 cases of acute myeloid leukemia [58]. *FLT3*-internal tandem duplication was also detected both at diagnosis and relapse of a case of acute myeloid leukemia with chromosome 13 UPD [59]. The high frequency of UPDs in acute myeloid leukemia has been confirmed by other research groups [60]. In basal cell carcinoma, LOH at 9q21-31 was found in 13 of 14 samples and 5 of the LOH are caused by UPD. Patched 1 gene (*PTCH*) in this region was found mutated in more than half of cases with LOH [8]. These studies demonstrated that mitotic recombination can act as a "second hit", similar to chromosome deletion, and responsible for removal of the remaining wild-type allele of those tumor suppressor-like genes. Using 10K SNP arrays, we also found a high frequency of large-scale homozygous chromosome regions (HCR) frequently extending to the whole chromosome without loss of chromosome copy number in the nonseminomatous subtype of male germ cell tumors. The frequency of these large-scale HCRs without chromosome copy number losses is significantly higher than the seminomatous subtype of germ cell tumor and the other tumor types, indicating its specific relationship with non-seminoma development [6]. Fig. (**2**) and (**3**) present our combined analysis approach and the high frequency of large-scale HCRs without chromosome losses. Since then, UPD has been found using SNP array analysis by other research groups in malignant lymphoma [61], myeloma [62], hepatocellular carcinoma [63] and colorectal cancers [64]. UPD is prevalent in myeloma [62] and half of the LOH regions in colorectal cancers are present as UPD and predominantly involve 8q, 13q and 20q [64]. In the colorectal cancer study, UPD did not change the expression level of genes in the relevant regions [64]. The correlation between DNA content and gene expression in the same type of cancer was also demonstrated by Tsafrir and colleagues [65] and in hepatocellular carcinoma by Midorikawa *et al.* [63].

Linkage Analysis for Cancer Susceptibility Loci

As SNP arrays can analyze genome-wide constitutional DNA polymorphisms in individuals, it is applicable for linkage analysis to identify cancer predisposition genes. In 2004, using the 10K array, Schaid *et al.* first validated the application of SNP array in linkage screen for cancer-susceptibility loci. Although they found that SNP array analysis can inflate LOD scores due to the presence of linkage disequilibrium, clearly, they demonstrated that SNP array analysis largely increased the linkage information content [66]. Causing inflated nonparametric linkage by the presence of high linkage disequilibrium was confirmed by Sellick and colleagues in a separate SNP array linkage analysis of chronic lymphocytic leukemia [67]. Despite this, Sellick *et al.* found some susceptibility loci such as 11p11, 5q22-23, 6p22 and 10q25 in chronic lymphocytic leukaemia. In colorectal cancer patients, Kemp *et al.* identified a disease susceptibility region of 3q21-24 which linked to the majority of families they analyzed [68]. Using 10K SNP arrays, Hu *et al.* [69] identified 37 SNPs associated with esophageal cancer and applied these 37 SNPs using principle components analysis to predict patients and normal individuals at more than 90% accuracy. Cao *et al.* [70] using SNP array analysis identified a 7 cM region on 10q23 with linkage to hereditary mixed polyposis syndrome, a disease which eventually leads to colorectal cancer. Subsequently, in one family they identified an 11 bp deletion in the bone morphogenesis protein receptor 1A (*BMPR1A*) gene. In pituitary adenoma, by screening a family using SNP array technology, Vierimaa *et al.* mapped a susceptibility locus to a previously reported chromosome region, 11q12–11q13. Further expression array analysis led to the selection of *AIP* gene for mutation analysis and identification of this low-penetrance tumor-susceptibility gene [71]. SNP array analysis has also been used to analyze the polymorphism of genes in xenobiotic metabolism and some SNPs in those genes have been associated with increased or decreased risk of colorectal cancers [72].

Other Applications of SNP Array Analysis in Cancer Research

Although SNP arrays were originally developed for genomic analysis, they were also adopted for epigenetic and gene expression analysis. As the current principle of Affymetrix array analysis is to apply selected restriction enzyme digested DNA samples for hybridization, it can be easily adjusted for DNA methylation studies by comparing the presence and absence of DNA sequences between the methylation sensitive and non-sensitive enzymes. Yuan and colleagues validated this application in decitabine treated leukemia and tumor/normal comparison of Wilm's tumor using bisulfite restriction analysis and bisulfite PCR as confirmation tools [73]. At the same time, application of the GoldenGate SNP array system for methylation analysis was validated at Illumina with the ability to distinguish lung cancer samples from controls [74]. DNA methylation can lead to differential expression of genes between paternal and mater-

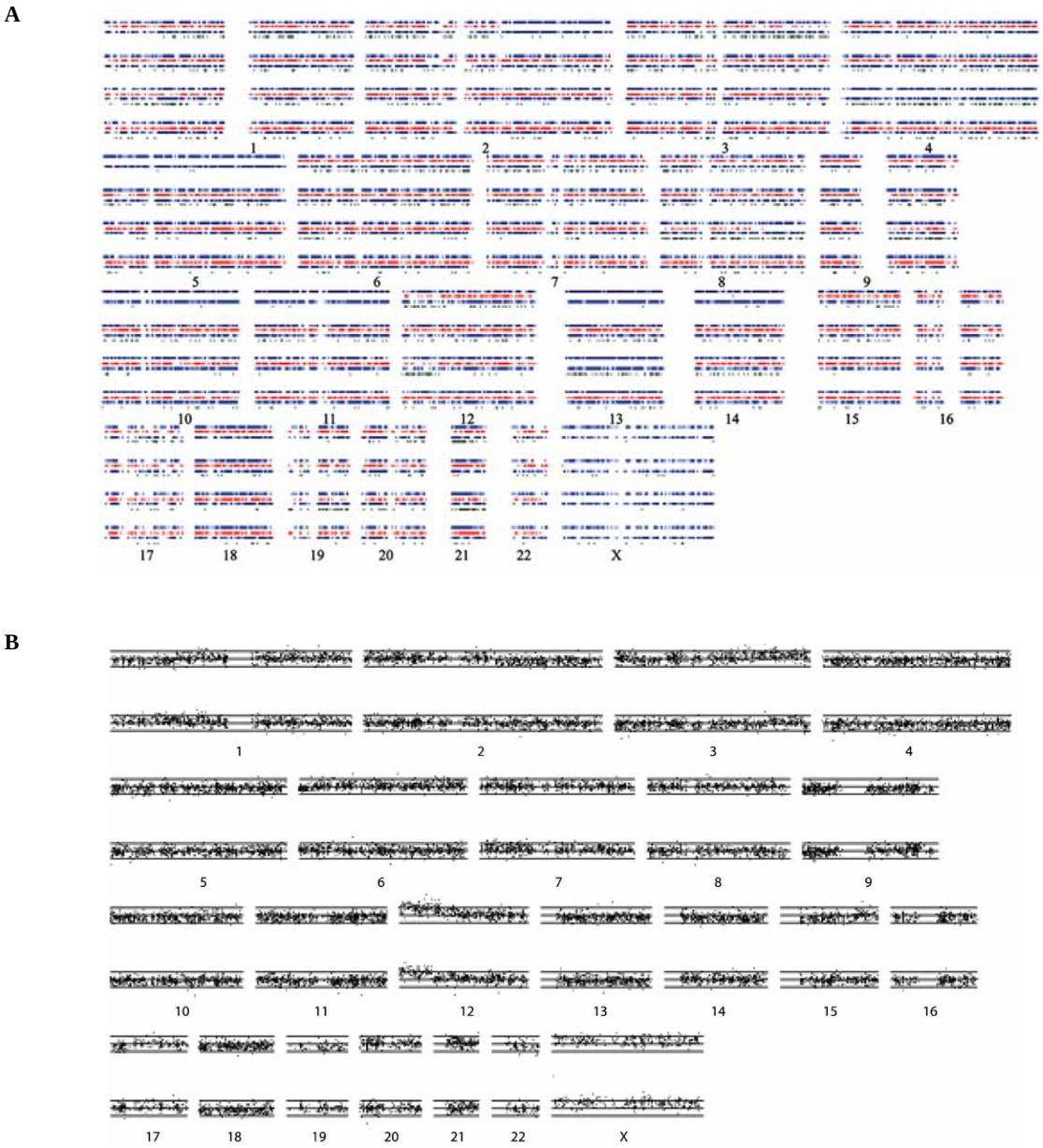

Fig. (2). Parallel genotyping and DNA copy number change analysis of testicular germ cell tumor. **A).** SNP genotype patterns along the chromosomes of two cases of non-seminomas with paired normal controls. From top to bottom, the cases presented are 5T (tumor), 5N (normal), 7T and 7N. It shows acquired large homozygous chromosome regions (HCRs, chromosome regions with 98% or greater homozygosities in a minimum region of 50 contiguous SNPs) on chromosome 2q, 5, 10, 11, 13 and 14 in case 5T and chromosome 4, 8q and 13 in case 7T. For each chromosome, two blue lines represent either AA or BB homozygous calls. The red line represents AB heterozygous calls and the grey line on the bottom shows SNPs not called. **B).** DNA copy number changes along the chromosomes of the same two tumors. The signal intensity of the tumor samples is normalized against the normal controls. The top case is 5T and the bottom case is 7T.

nal alleles, which is referred to as DNA imprinting. Other factors such as DNA polymorphisms in gene regulatory factors can also cause imbalanced allelic expression [75]. Using cDNA instead of genomic DNA as starting material, allelic specific gene expression can also be detected by SNP array analysis [76, 77]. Allelic specific gene expression may be important in tumorigenesis. However, allelic specific gene expression pattern analysis directly using SNP arrays has not been explored in the field of cancer research.

ADVANTAGE AND LIMITATION OF SNP ARRAY ANALYSIS

The advantage of SNP array in high throughput DNA genotyping over PCR based individual microsatellite marker polymorphism analysis is obvious and has been demonstrated in papers summarized above. Compared with other array technology, the high density resolution of SNP arrays is clearly an advantage. It is estimated that there are over 20

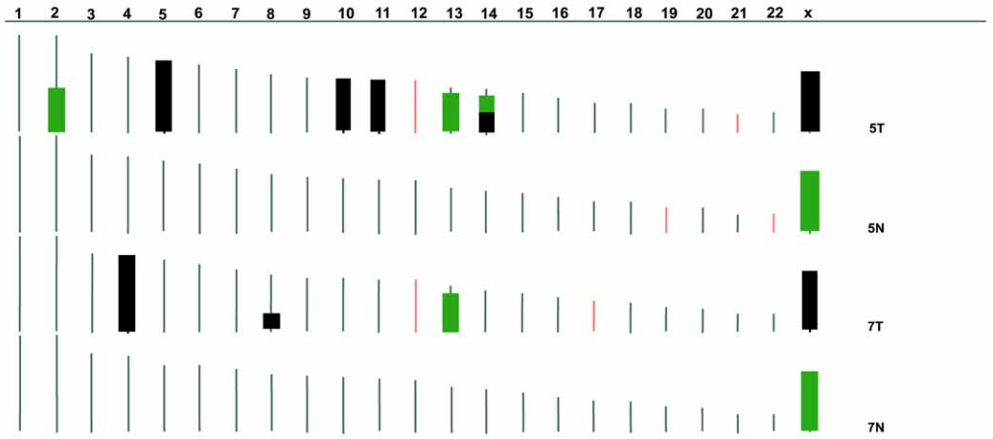

Fig (3). A summary of the HCRs on each chromosome for the two pairs of samples analyzed by SNP arrays. Black bars are HCRs without significant chromosome copy number changes (signal intensity ratio compared to case average between 0.75-1.25); green bars are HCRs with relative chromosome copy number loss (signal intensity ratio < 0.75).

million SNPs in the entire human genome, on average every 150 base pair per SNP. The whole-genome genotyping SNP array technology under current development has the potential to comprise almost all existing SNPs into a single experiment. The resolution of genetic abnormalities picked up by high density SNP array analysis will be more than sufficient for direct PCR and sequencing analysis. The unique feature of SNP arrays, and the concurrent analysis of both genotype and DNA copy number changes, make SNP arrays irreplaceable by any other high throughput technology.

However, every technique has its limitations. Similar to the other DNA array-based methods, SNP array analysis cannot detect balanced chromosomal translocations, inversions and whole-genome ploidy changes without combination with other techniques. Therefore, SNP array analysis of DNA extracted from a cell population cannot indicate the heterogeneity within the sample. In this respect, combination of data with those from individual cell analysis methods such as FISH or 24-color FISH karyotyping is necessary. Although SNP arrays can be used for allelic specific gene expression analysis, they are principally designed for genomic study. More specific expression arrays such as oligonucleotide and exon array are required for transcription level study. Further functional studies should also be correlated with genomic and expression alterations. In the case of evaluating known genes or established genetic markers in a large series of samples, PCR and tissue array analyses will be more efficient.

CONCLUSION

SNP array technology is a powerful genomic analysis tool. It concurrently generates data identifying genome-wide genotyping and DNA dosage alterations. The application of SNP arrays in combination with PCR, tissue array and functional analysis technologies will eventually lead to the full revelation of the complex genetic alterations in human cancers.

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ABBREVIATIONS

SNP	=	Single nucleotide polymorphism
FISH	=	Fluorescence *in situ* hybridization
CGH	=	Comparative genomic hybridization
LOH	=	Loss of heterozygosity
PCR	=	Polymerase chain reaction
AI	=	Allelic imbalance
SSLP	=	Simple sequence length polymorphism
UPD	=	Uniparental disomy
HCR	=	Homozygous chromosome region

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