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# MOLECULAR PROFILING AND GENOMIC MICROARRAYS IN PROSTATE CANCER

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**In the present review article a global approach regarding the usefulness of genomic microarrays in prostate cancer management, is attempted. Cancer is a multistep process of mutations in key regulatory genes and epigenetic alterations that result in loss of balanced gene expression. A complete knowledge of the interaction between the genetic variability of the neoformation (tumor profiling) and the genetic variability of the host (inherited genome profiling), will be able to determine the better strategy against the cancer and the less toxicity for the patient. Alterations in the sequence of the hormone binding domain of the androgen receptor as well as mutations in some genes, determine radioresistance and resistance or sensitivity to some chemotherapeutic drugs. New therapies using monoclonal antibodies directed against specific extracellular binding domains of some receptors are based on molecular alterations observed in tumors.**

*Key Words:* **prostate cancer, microarrays, genomics.**

Genomic arrays are based on the large scale analysis of the genome information to predict prognosis, for a better management of the disease and to identify molecular targets in order to discover new drugs.

Cancer is the result of a multistep process of mutations in key regulatory genes and epigenetic alterations that result in loss of balanced gene expression. A complete knowledge of the interaction between the genetic variability of the neoformation (tumor profiling) and the genetic variability of the host (inherited genome profiling), will be able to determine the better strategy against the cancer and the less toxicity for the patient.

## TUMOR PROFILING

Many tumors harbor somatic mutations which make them resistant to therapy. For example: paradox prostate cancer responses to antiadrogens or glucocorticoids in the "androgen withdrawal syndrome" have been related to pharmacogenetic alterations in the sequence of the hormone binding domain of the androgen receptor; mutations in the tubulin gene determine resistance to chemotherapeutic drugs such as vinblastin, etoposide or taxanes. Radioresistance has been associated with overexpression of the EGR-1 gene [1]. Moreover, the status of the target in the neoplastic mechanism is particularly important for new therapies using receptor ligands such as endothelin-1 antagonists or monoclonal antibodies such as cetuximad or trastuzumab which are directed against specific extracellular binding domains of the growth factor receptors EGFR (Erb B1) or Her-2/Neu (Erb B2), respectively. Other molecular alterations in tumors have been associated with drug resistance or sensitivity. They are not restricted to a specific drug because they involve the multidrug resistance gene family (MDR 1) or ubiquitous genes (p53, bcl-2, p21, p14, topoisomerases I and II, which regulate cell cycle proliferation), apoptosis or DNA repair [2]. The number of molecular alterations observed in tumors and genetic complexity which could determine therapeutic efficiency in a global fashion has suggested using high-density microrrays in order to generate tumor profiling from thousands of gene patterns [1, 2].

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## HIGH-DENSITY ARRAYS

In urologic oncology, the use of expression profiling for diagnosis, classification and outcome prediction was recently demonstrated using micro arrays on prostate and other cancers of the uro-genital apparatus. Because genetic or epigenetic events can be important in malignant progression or drug response, the identification of large scale genomic or protein profiling could be promising [3]. Tumors and particularly prostate cancers are heterogeneous mixtures of different cell types which can complicate the interpretation of genomic analysis [1]. Because of this heterogeneity, sample selection is an important issue and needs careful histopathologic examination of specimens before microarrays analysis [3]. In some cases, laser capture micro dissection techniques combined with the DNA, RNA or protein chip technology allows for the isolation of selected cells from a tissue section and has been used to isolate specific and homogeneous cell types. Mapping of cancerous foci, blood vessels or immunocompetent cells in malignant tissues can be used to identify tissue-specific markers that serve as potential targets for *in vivo* drug delivery [4].

However, apart from the fact that microdissection reduces the scope of dissection of biological events in a single choice cell type and for example occults interaction between different cell compartments in pathological tissues, it is difficult to obtain adequate

amounts of high quality RNA for expression profiling [2]. Microarrays usually require between 10 to 40 μg (2 μg at least for radio-labeled nucleic acid) of high quality RNA, corresponding roughly to a 100 $mm^3$ piece of tissue. To obtain high quality RNA, tissue samples should be snap-frozen in liquid nitrogen within half an hour of trans-rectal or trans-urethral multiple biopsies of the gland and stored at –80 °C. Unfortunately, and contrary to DNA, methods do not yet exist for obtaining sufficient mRNA from formalin-fixed tissues [1, 2].

Microarray slides are shared in two groups depending on the type of nucleic acid spotted onto the slide and the method used to array and spot it: i) micro arrays manufactured with *in situ* synthesized oligonucleotides between 25 to 80 mers; ii) spotted nucleic acids (with pin tool or ink jet technology) including long oligonucleotides (80 mers) or DNA fragments (cDNA, genomic DNA). Whereas the two types of micro arrays could be used for gene expression, oligonucleotide micro arrays are usually chosen for detecting point mutations, and DNA fragments micro arrays could be used for genomic integrity testing by decrypting chromosome alterations (comparative genomic hydridization array, CGH array) [5].

Using DNA or array based comparative genomic hydridization (aCGH) in prostate cancer is able to finely map chromosomal deletions (and amplifications) in tumors. Using a CGH, more additional subtle genomic changes, like amplicons or homozygous deletions, are detected in the prostate cancer genome than using cytogenetic or classic CGH techniques and demonstrate the viability of a CGH for mapping regions of chromosomal aberrations [5, 6].

aCGH represents an advance beyond metaphase CGH, and is a more sensitive, higher resolution technique. Standard CGH has been informative, however the limit of resolution is 10–15 Mb, making fine mapping of regions impractical [5]. In aCGH, chromosomal loci with copy number changes are linked to the human genome sequence by the genomic coordinates of BAC clones on the array, defining amplifications or deletions with resolution limited only by the spacing of BAC clines on the human genome. Thus aCGH can both more accurately define subtle aberrations undetectable using standard CGH, and resolve the boundaries of known regions [3, 5]. The data derived from aCGH analysis can be clustered, similar to gene expression profiling, thus providing a powerful method to cluster tumors [4, 5].

Different methods using RNA (cDNA) on array are available for large scale gene expression analysis [2]. Some of them are powerful as they are completely open systems (no knowing material is required) like differential display or serial analysis of gene expression (SAGE) but they are technically heavy and very expensive [3, 5]. Microarray techniques belong to semi-closed systems, because sequences of immobilized nucleic acids should be known. This technology came to light almost ten years ago with the first addressable *in vitro* synthesis of oligonucleotides onto a solid surface leading to the first application in discriminating human pathologies based on the expression of sets of genes [7].

Microarray experiments generate thousands of data which need organization, storage and analysis. Currently, there are no or few standards to design or compare databases on micro arrays results. However, at investigation of the Microarray Gene Expression Database (MGED) group, several research groups tend to define the minimal criteria required for any publication of results based on microarray experiments, and have drawn up the Minimum Information About Microarray Experiment (MIAME).

## "AD-HOC" DENSITY ARRAYS

Microarray technology constitutes a powerful tool for high-throughput analysis of biological or clinical specimens. Technical advance combined to decreasing costs contribute to bring microarrays accessible, commercially available whole genome arrays commonplace. Controversially, beyond the initial enthusiasm that any new technology can induce and the fascination that researchers or clinicians have in front of thousands of genomic data from a single sample, their clinical aptness to become a routine tool start just to be demonstrated. Considerations about clinical applications such as tissue sampling and biological material variability and data analysis, still present major limitations for routine use [8].

Consequently, molecular profiling have been developed using a set of 10 to 200 "ad-hoc" genes of which the pattern of alteration is the most discriminating for the management of the disease [6, 8]. Genotyping, real-time RT-PCR or tissues arrays are adapted for this approach dedicated to clinical validation of results obtained by large scale or whole genome screening and to turn on individual genomic medicine.

## INHERITED (GERM LINE) GENETIC PROFILING

Therapy is often limited to a certain cumulative dose. If practitioners could identify patients with genetic factors that are associated with a lower probability of drug or beam therapy toxicity, they would be able to administer higher, more effective doses to such patients, and thereby increase the therapeutic range for that subset of the patient population. Moreover, for prostate cancer genetic polymorphisms and particularly those involving genes determining the steroid biosynthesis and metabolism pathway (androgen receptor, 5-alpha-reductase type II, CYP 17, aromatase, CYP 3A4, Cyp1B1, vitamin D receptor genes) or drug and carcinogen metabolizing enzymes (NAT 1 and NAT 2, CYP2D6, CYP3A, CYP1B1, glutathione-5-transferase) or DNA repair genes (ATM, XRCC1, XRCC3, XRCC5, NBS, MRE11, ARE1, RAD50) or oxidative stress (SOD2) or post radiotherapy fibrosis (TGF beta-1) were hypothesized to be the probable explanation for differences in tumor aggressiveness, therapeutic response and adverse effects of treatment among genetic variability [9, 10, 11, 12]. Thus, genomic

investigations of constitutional DNA changes in human genes predisposing to cancer may lead to significant advances in management of prostate cancers but need also large scale genomic profiling [10, 13, 14, 15].

## CONCLUSION

It is clear that genomics arrays continue to be applied with success in many relevant areas of research and are ripe for application to a multitude of clinical problems especially in oncology. However, there are substantive technical issues associated with the use of DNA microarrays data that limit their interpretation and consequently their implication in clinical practice. The true challenge of the future for clinical routine use of DNA microarrays will be to determine if clinician need genome-wide gene alteration profiling or a limited and relevant set of genomic data specifically designed for dedicated disease or therapeutic strategy.

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# МОЛЕКУЛЯРНЫЕ ОСОБЕННОСТИ КЛЕТОК РАКА ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ

**В обзоре обсуждается целесообразность применения геномных микрочипов для выявления рака предстательной железы. Рак является многоэтапным процессом мутаций в ключевых регуляторных генах и эпигенетических изменений, приводящих к утрате сбалансированной экспрессии генов. Фундаментальные знания о взаимосвязи между генетической вариабельностью опухолевых клеток (молекулярном профиле опухоли) и генетической вариабельностью хозяина (наследуемый геномный профиль) позволит выбрать наилучшую стратегию противоопухолевой терапии при низкой токсичности таковой. Изменения последовательности гормонсвязывающего домена рецептора андрогена наряду с мутациями некоторых генов определяют устойчивость к лучевой терапии и устойчивость или чувствительность к ряду химиопрепаратов. Новые виды терапии с использованием моноклональных антител против специфичных внеклеточных связывающих доменов ряда рецепторов основаны на данных о молекулярных особенностях новообразований.**

***Ключевые слова:*** **рак предстательной железы, микрочип, геномика.**