

## Microarray in the Direction of Oral Cancer- A Review Paper

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### Review Article

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**Abstract:** Cancer is a highly variable disease with multiple hetero-geneous genetic and epigenetic changes. Current cancer diagnosis and classification relies on clinical and histopathological information. However, some cases bring diagnostic confusion due to incomplete clinical information and atypical histopathologic features. The final deciphering of the complete human genome, together with the improvement of high throughput technologies, is causing a fundamental transformation in cancer research. Microarray technology permits simultaneous analysis of thousands of DNA sequences for genomic research and diagnostic applications. Microarray helps in studying the molecular basis of interactions on a scale that is impossible using conventional analysis. This technology promises to lead to improvements in developing rational approaches to therapy as well as to improvements in cancer diagnosis and prognosis. Predicting who will develop cancer and how the disease will behave and respond to therapy after diagnosis will be one of the potential benefits of this technology.

**Keywords:** Microarray, cDNA, Oral Cancer

### INTRODUCTION

The term karkinos was coined by Hippocrates for breast cancer. The term cancer means crab since it sticks to the part stubbornly like a crab [1].

Cancer is defined as a disease of cellular proliferation that is malignant and primary characterized by uncontrolled cellular proliferation, local cell invasion and metastasis [2]. It is a multigene, multistep disease originating from a single abnormal cell with an altered DNA sequence [3]. Uncontrolled proliferation of these abnormal cells is followed by a second mutation leading to the mildly aberrant stage. Successive rounds of mutation and selective expansion of these cells results in the formation of tumour mass [3].

The 6 hallmarks of cancer currently accepted [3]:

- Immortality : Continuous cell division and limitless replication
- Produce “GO” signals(growth factors from oncogenes)
- Override “STOP” signals (anti-growth signals from tumour suppressor genes)
- Resistance to cell death(apoptosis)
- Angiogenesis: introduction of new blood vessel growth
- Metastasis : spread to other sites

The common term used for all malignant tumours is cancer.

Microarray can be defined as an ordered collection of microspots (the probes), each spot containing a single species of a nucleic acid and representing the genes of interest [4]. The term

microarray was first introduced by Schena *et al.*, [5] in 1995 and the first genome of an eukaryotic species completely investigated (*Saccharomyces cerevisiae*) by a microarray was published in 1997 [6,7]. The microarray is powerful molecular technology that allows simultaneous study of expression of thousands of genes or their RNA products [4]. DNA microarray helps in determining whether the DNA from a particular individual contains a mutation in genes like BRCA1 and BRCA2 [8]. Microarrays could be used in combination with other diagnostic methods to add more information about the tumor specimen by looking at thousands of genes concurrently [9]. The microarrays have become important because they are easier to use, do not require large-scale DNA sequencing and allow the parallel quantification of thousands of genes from multiple samples [4].

**DISCUSSION**

**Variants of microarrays**

There are mainly two variants of micro- arrays:

- cDNA microarrays and
- Oligonucleotide microarrays
- Although both types of microarray are used to analyse gene expression patterns, these variants are fundamentally different

**In cDNA microarrays**

In cDNA microarrays relatively long DNA molecules are immobilized on a solid surface. This type of microarray is mostly used for large-scale screening and expression studies [10].

**Advantages of cDNA microarrays [11]**

- Relative affordability with a lower cost
- Its accessibility requires no specific equipment for use such that hybridisation does not need specialised equipment.
- Data capture can be carried out using equipment that is very often already available in lab.

**Disadvantages of cDNA microarrays [11]**

- Intensive labour requirement

- May hybridise to spots designed to detect transcript from a different gene.

**The oligonucleotide microarray**

The oligonucleotide microarray is fabricated by in situ light-directed chemical synthesis or by conventional synthesis followed by immobilization on a glass matrix. This microarray is used for detection of mutations, gene mapping and expression studies and allows for the differential detection of gene family members or alternative transcripts that are not distinguishable by cDNA microarrays [11].

**Advantages of oligonucleotide microarray [11]**

- Speed
- Specificity
- Reproducibility

**Disadvantages of oligonucleotide microarray [11]**

- Tend to have expensive specialised equipments.
- Short sequences used on the array have decreased sensitivity/binding compared with cDNA microarrays.

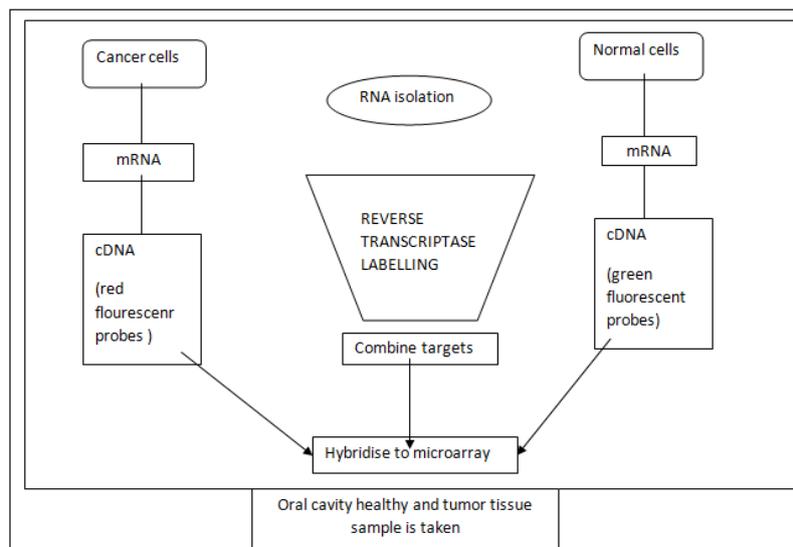
<b>ISOLATION</b>	Silicamembrane based isolation of nucleic acids or protein isolation
<b>LABELING</b>	Incorporation of fluorescent dyes
	Use of streptavidin modified primers or Adding of fluorophores to proteins
<b>HYBRIDISATION</b>	Blocking of arrays
	Hybridization
	Washing of arrays
<b>DETECTION</b>	Generation of array images using fluorescent microarray scanner
<b>DATA EXTRACTION</b>	Extracting signal intensities from images
<b>BIOINFORMATIC</b>	Data normalisation and statistical data analysis

**Steps in microarray experiment [7]**

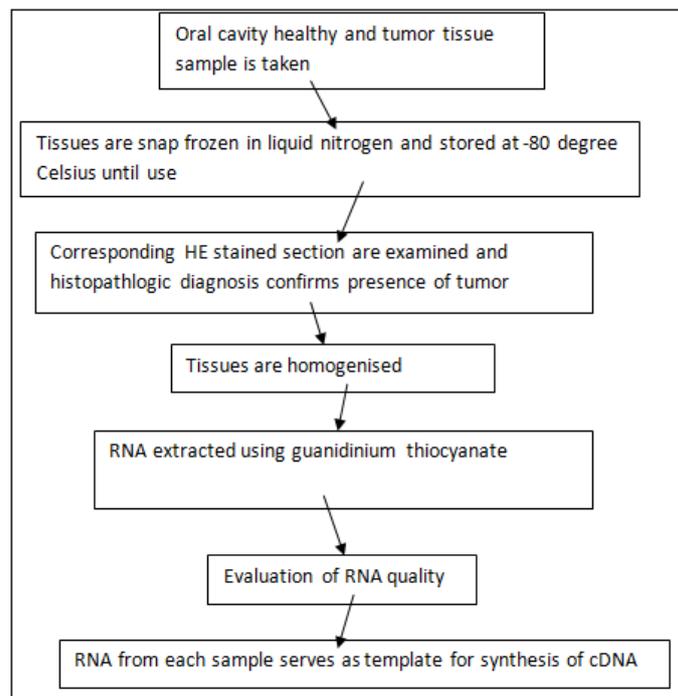
**Working of DNA microarray [13]**

In each type of cell, like a muscle cell or a skin cell, different genes are expressed (turned on) or silenced (turned off). If the cells that are turned on mutate, they could—depending on what role they play in the cell—trigger the cell to become abnormal and

divide uncontrollably, causing cancer. By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer. One way they do this is to use a DNA microarray to determine the expression levels of genes.



Following are the steps for sample collection, sample storage and RNA isolation



When a gene is expressed in a cell, it generates messenger RNA (mRNA). Overexpressed genes generate more mRNA than underexpressed genes. This can be detected on the microarray. The first step in using a microarray is to collect healthy and cancerous tissue samples from the patient. This way, doctors can look at what genes are turned on and off in the healthy cells compared to the cancerous cells. Once the tissues samples are obtained, the messenger RNA (mRNA) is isolated from the samples. The mRNA is color-coded with fluorescent tags and used to make a DNA copy (the mRNA from the healthy cells is dyed green; the mRNA from the abnormal cells is dyed red.)

#### How DNA Microarrays Work [13]

The DNA copy that is made, called complementary DNA (cDNA), is then applied to the microarray. The cDNA binds to complementary base pairs in each of the spots on the array, a process known as hybridization.

Based on how the DNA binds together, each spot will appear red, green, or yellow (a combination of red and green) when scanned with a laser.

- A **red spot** indicates that that gene was strongly expressed in cancer cells.

- A **green spot** indicates that that gene was strongly repressed in cancer cells.
- A **yellow spot** indicates that gene was neither strongly expressed nor strongly repressed in cancer cells.
- A **black spot** indicates that none of the patient's cDNA has bonded to the DNA in the gene located in that spot. This indicates that the gene is inactive.

#### Application of microarrays in oral cancer

Head and neck squamous cell carcinoma (HNSCC) is second only to lung cancer as the most common smoking-related cancer worldwide. Oral squamous cell carcinoma (OSCC) is the most common anatomic site of HNSCC counting for approximately 50% of all HNSCC. Despite the tremendous effort to reduce tobacco use, HNSCC remains one of the leading causes of smoking-attributable mortality in the world—about 438,000 each year. Even for HNSCC diagnosed at early stages, surgery (current standard care) is a debilitating, substantially morbid procedure that severely impairs quality of life for many patients.

#### Other important applications of microarrays in dentistry

Increase in the number of resistant bacteria and superadded infections have led to failure of antibiotics. In the oral cavity where anaerobic bacteria might be infective agent, they often are not easily culturable particularly actinomyces. DNA microarray helps as bacterial genomic DNA often outlasts viability of bacteria and a diagnosis can be made using small amount of DNA. In future, an abscess specimen need not to be sent for culture and sensitivity testing but will be sent for microarray analysis.

#### OTHER USES OF MICROARRAY

- Used for comparative genomic hybridisation. It has been used to map genetic abnormalities in a wide range of tumours, including breast carcinoma, fallopian tube carcinoma, bladder carcinoma, gastric carcinoma, melanoma and lymphoma.
- Disease diagnosis
- Drug discovery
- Toxicological research

#### Limitations of microarrays [7]

- Since microarray experiments need the physical disruption of a cell to gain access to its gene expression patterns; this technology can be considered as an example of 'destructive testing'.
- There is also a lack of rigorous standards for data collection, analysis and validation.
- The quality and amount of RNA remains a major challenge in the microarray experiments. The amount of obtained tissue and the complexity of the tissue sample itself limit the quality and quantity of RNA that can be isolated. Therefore, clinical studies that are published using the microarray approach are performed in settings

where biological samples are abundant and easily obtainable.

- Processing tissue rapidly to maintain RNA integrity is crucial. False microarray data can be generated from degraded mRNA.
- Owing to the numerous error-prone steps in microarray experiments, the experiments need to be replicated in order to eliminate sources of error.
- Furthermore, the small size of many clinical specimens from early diagnoses is another critical problem. Efforts are under way to reduce the amount of sample required for analysis. Most microarray platforms work with a few micrograms of mRNA, except for nylon microarrays, which use only a few nanograms of nucleic acid [14].

#### CONCLUSION

In light of its continuing burden and evasion of substantial control, HNSCC requires new approaches like microarrays will be helpful in the diagnosis of the disease before the cancerous stage and preventing development of invasive cancers [15]. Microarray can be a boon to researchers as it provides a platform for simultaneous testing of large set of genetic samples. It helps in identification of single nucleotide polymorphisms (SNPs) and mutations, classification of tumours, identification of target genes of tumour suppressors identification of biomarkers, identification of genes associated with chemoresistance and drug discovery. Most human tissue samples are a mixture of different cell types. Therefore, changes in gene expression patterns, when comparing two different tissue biopsy samples, are a manifestation of all the cell types present in that sample. This issue can render the analysis inaccurate. Methods such as laser capture microdissection, which permits the isolation of specific cells, can be useful but still are limited by technologically and expenditure [16,17,18,19].

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