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Utilization of microarray analysis to determine therapeutic targets in human cancers

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Abstract

Advances in DNA microarray technology have significantly improved research-based therapeutic outcomes. The rapid determination of gene expression profiles in malignant and nonmalignant cells and tissues have paved the way for the elucidation of beneficial molecular details regarding the development and progression of human diseases and the design of biomarkers. This review article focuses on microarray studies of human cancers from primary cells and cell lines. Signal transduction pathways and genetic factors that may have significant therapeutic and prognostic value are also examined in this comprehensive review.

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Introduction

Technological improvements in the last twenty years have significantly enhanced our ability to elucidate the underlying molecular mechanisms that promote essential biological processes. Of the many sophisticated scientific instruments and elegant experimental protocols, no other technology has improved our understanding of critical intracellular genetic interactions more significantly than microarray technology (Hackl *et al.*, 2004). DNA microarray technology offers a great deal of promise in the areas of drug design, chemotherapeutic optimization, signal transduction research, gene expression profiling, biomarkers, molecular pathology, genetic control mechanisms, and numerous other areas of biomedical research (Macgregor and Squire, 2002; Smith *et al.*, 2013; Xu *et al.*, 2013).

The synergy of microarray studies and bioinformatics analysis will positively affect research investigations into the genetic causes of human cancers. Bioinformatics is the science of identifying the structure and function of genes and proteins using computer-assisted analysis. Bioinformatics is an interdisciplinary science that combines areas of biology, mathematics, computer science, and other disciplines (Arvind, 2005). Bioinformatics analysis of genetic and proteomic information will lead to a more detailed understanding of the molecular basis of key biological processes such as cellular metabolism, human disease, host-microbe interactions, inflammation, cell cycle regulation, and evolution. The study of various genes and proteins using bioinformatics may serve to predict future chemotherapeutic agents and vaccine candidates for cancer (Hardy and Singleton, 2009). Critical to the success of interpreting microarray data is the utilization of accurate bioinformatics software. Common commercial pathway analysis software programs include Pathway Studio, Ingenuity Pathways Analysis, and GeneGO as well as open source software platforms such as Cytoscape and PathVisio. Bioinformatics software is instrumental in elucidating molecular networks, related diseases, and associated biological processes that correspond with the dataset under investigation.

There have been many studies that have utilized DNA microarray technology and other genomic analysis procedures to elucidate genetic factors that promote health and disease (Chen and Wang, 2012; D'Angelo *et al.*, 2014; Goyal *et al.*, 2013). To determine the genetic influences of health disparities linked to different types of carcinomas Perrone *et al.* (2000) collected biological samples from clinical study participants from distinct racial communities with varying clinical diagnoses in order to elucidate trends in gene expression patterns following microarray analysis.

Similar experimental approaches will allow physicians and researchers the opportunity to monitor and track disease progression and identify specific genomic trends that may pinpoint genes or gene families that account for differential mortality rates. In this review article we explore the experimental evidence that clearly supports the use of DNA microarray technology to unravel molecular details of human cancers. By studying the gene expression patterns of various human cancers, oncologists and biomedical scientists may better predict a patient's response to surgical and chemotherapeutic treatments (Macgregor and Squire, 2002).

In addition, comparison studies in which normal tissue and cancer tissue are subjected to microarray analysis will lead to novel approaches to treat cancer and supplant existing knowledge in the field of cancer biology and molecular genetics. Recent DNA microarray comparison studies in our laboratory involving DU145 cells, a human prostate cancer cell line, and normal primary prostate epithelial cells have generated exciting results regarding differential gene expression profiles for the two distinct cell types and may offer new molecular clues regarding prostate cancer development and progression. Additionally, microarray comparison studies in which racial differences, gender differences, age differences, and

differences in treatment methods (e.g., drug dosage, radiation exposure) are compared will continue to elicit beneficial data that may help reduce health disparities and provide critical biological explanations to improve treatment options. These studies may also offer a unique understanding of genetic interactions and potential proteins that may explain why some treatment strategies work better than others for specific demographics.

It is important for biomedical researchers to note however, that cancerous tissue is not homogenous but rather a heterogeneous composition of cells and that gene-expression data produced from microarray experiments and subsequent bioinformatics analysis from one cellular subtype derived from a tumor may not provide similar oncogenic properties when compared with another cellular subtype from the same tumor (King and Sinha, 2001).

Signal Transduction Mechanisms

Many studies have clearly confirmed the importance of a variety of signal transduction mechanisms in mediating normal cellular functions. Moreover, within the last 20 years signal transduction research has also implicated numerous signaling pathways in a copious amount of human diseases and pathophysiological effects associated with the dysregulation of signaling molecules such as transcription factors, kinases, and hormones (Flowers, 2013). A highly studied signal transduction pathway that has been extensively used to elucidate potential biomarkers and causes of malignant transformation of human cells is the Janus Kinase-Signal Transduction and Activators of Transcription (JAK-STAT) pathway.

The JAK-STAT pathway is mediated by extracellular binding of cytokines, growth factors, and hormones and converted to intracellular signals that induce gene-expression and biological function. The JAK-STAT pathway consists of four tyrosine kinase proteins (JAK1, JAK2, JAK3, TYK2) and seven transcription factors (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6) (Flowers, 2012).

Additionally, the HGF/c-Met signaling pathway is a signal transduction pathway that has recently been implicated in a wide variety of human carcinomas (Goyal *et al.*, 2013; Lim *et al.*, 2014; Yu *et al.*, 2012) and not surprisingly plays a role in cell proliferation and development. The c-Met oncogene specifically encodes the hepatocyte growth factor receptor (HGFR). HGFR is expressed in many different mammalian cell types and is stimulated by the hepatocyte growth factor (HGF). Binding of HGF to the c-Met tyrosine kinase receptor leads to receptor dimerization and autophosphorylation of the receptor.

Activation of additional signal transduction pathways (e.g., phosphoinositide 3-kinase and Cdc42/Rac1 pathways) are facilitated via Gab1/Grb2 adaptor proteins (Goyal *et al.*, 2013; Lim *et al.*, 2014). Cellular activation of the hepatocyte growth factor receptor (c-Met) leads to a variety of biological functions that are also associated with tumor development (Figure 1). Accordingly, mutations in c-Met or aberrant hepatocyte growth factor receptor function have malignant consequences (Peruzzi and Bottaro, 2006). Moreover, abnormal c-Met tyrosine kinase activity has been experimentally linked with metastasis, angiogenesis, and cancer cell survival (Longati *et al.*, 2001).

Further, aberrant c-Met tyrosine kinase activation has been shown to contribute to a wide variety of human cancers including, prostate cancer, renal cancer, neck cancer, and colon cancer. It is envisioned that effectively blocking the c-MET signal transduction pathway may serve as an effective therapeutic strategy for many human cancers (Bellon *et al.*, 2008).

Human Cancer and Microarray Analysis

In a recent study Limame *et al.* (2013) isolated migrating and invading cells from triple negative breast cancer tissue to determine the gene expression signatures of these cells. Results from the microarray analysis identified Kruppel-like factor 9 (KLF9) as a potential inhibitor of metastatic potential in breast

cancer cells. Moreover, the use of overexpression experiments utilizing an EGFP-KLF9 fusion protein attenuated invasion and growth of the MDA-MB-231 breast cancer cell line thereby validating expression data with a functional assay. In another study involving a transgenic mouse model of breast cancer, dose-dependent effects of quercetin were determined. Mammary glands of mice treated with 0.2% quercetin (moderate-dose) and no quercetin (control) were extracted to determine gene expression profiles. Steiner *et al.* (2014) identified 31 genes that were attenuated and 9 genes that were up-regulated by more than two-fold following quercetin treatment. An examination of tumor number and tumor size were also assessed. As mentioned earlier in the review article, the research study described above can be employed to determine precise gene expression signatures associated with therapeutic treatments. In these types of studies inspection of the specific altered genes may lead to novel treatments or additional molecular targets that could enhance the antitumor effects observed.

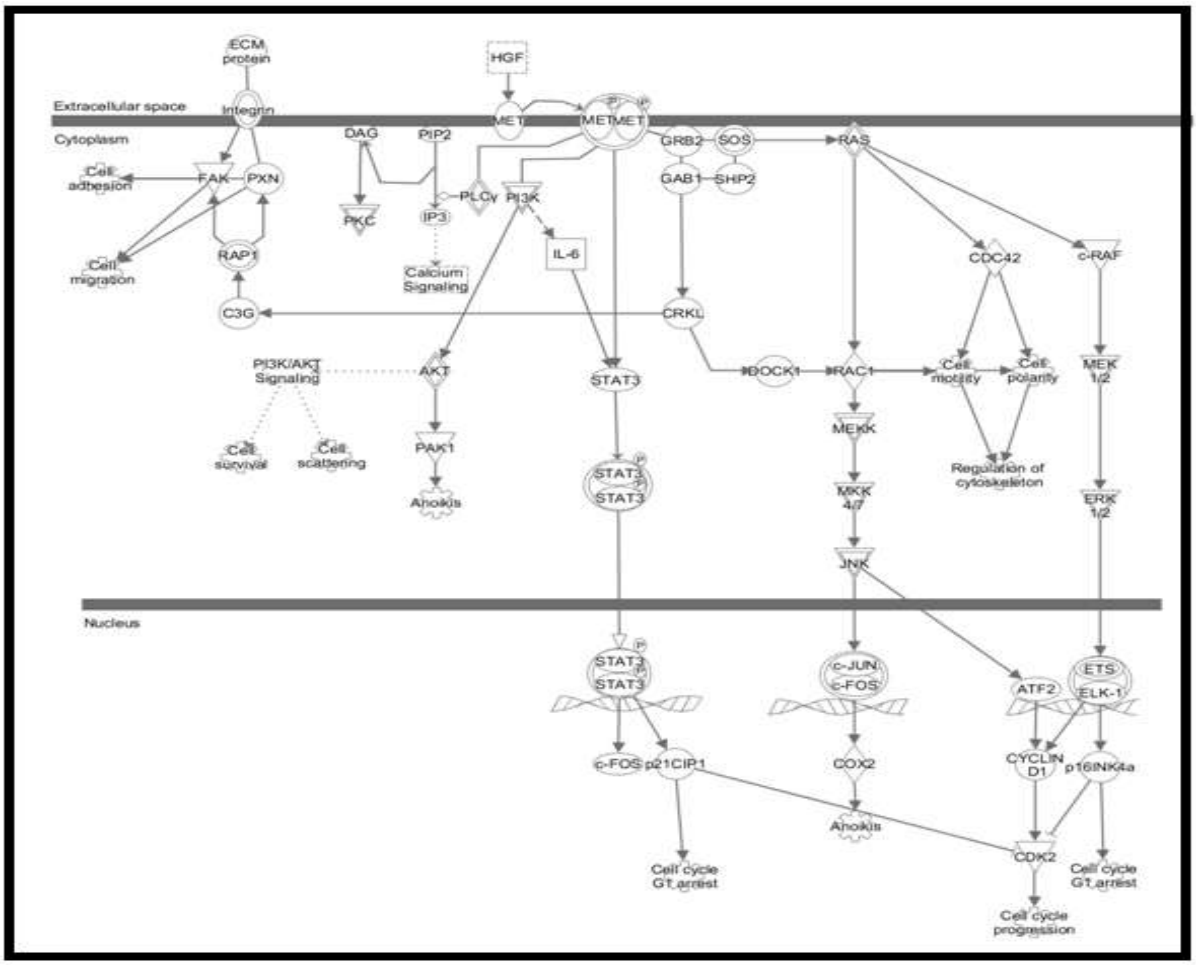

Fig. 1. HGF/c-Met canonical signaling pathway designed using Ingenuity Pathway Analysis (IPA). Shapes denote different biological molecules: cytokine (□), kinase (▽), phosphatase (△), peptidase (◊), transcription receptor (0), complex (◎), and other (○). Arrows correspond to the following relationships: acts on (A➔B), inhibits (A—IB), reaction (A➔B), direct interaction (—), and indirect interaction (----).

Invasive ductal carcinoma (IDC) is a very common type of breast cancer and constitutes a health threat to women around the world. Elucidation of the biological mechanisms that promote carcinoma development are essential in the development of therapeutic weapons to fight the disease. Huan *et al.* (2014) performed bioinformatics experiments using microarray datasets of breast cancer tissue samples and normal controls to screen for potential obligatory IDC genes. Fifty-six differentially expressed genes

were detected with the majority (51 genes) being up-regulated in IDC tissue. Analysis also revealed a network of diseases and pathways associated with the IDC gene signature. Pathways associated with nucleotide excision repair and ECM-receptor interactions were indicated following analysis using KOBAS (KEGG Orthology Based Annotation System) (Xie *et al.*, 2011). The identification of disease-associated pathways is essential for a complete understanding of the biological mechanisms involved in IDC and could serve as prospective pathways to explore during the drug design process.

Cancer stem cells (CSCs) are generally believed to be responsible for tumorigenic properties such as metastatic potential and tumor recurrence even though they constitute only a minor subset of tumor cells. Chen and Wang (2012) utilized microarray analysis to determine the gene expression signature related to prostate cancer stem cells. In their experiment, the cancer biology researchers isolated CSCs from DU145 human prostate cancer cells and compared the gene expression profiles of both cell types to identify genes and biological processes that play a role in the tumor-initiating properties exhibited by prostate cancer stem cells. A total of 231 genes were found to be differentially expressed in the CSCs (Chen and Wang, 2012). Lipopolysaccharide-induced TNF factor (LITAF) and natural killer-tumor recognition sequence (NKTR) are two genes that showed reduced expression in CSCs compared to DU145 cells and may play a role in promoting CSCs resistance to the human immune system as well as immunological therapeutic procedures. Overexpression studies involving LITAF and NKTR may be used to explore the efficacy of targeting these two genes to elicit antitumor effects. Biological processes such as metabolism, transcriptional regulation, and the cell cycle were implicated as a result of bioinformatics analysis and may serve as potential mechanistic sites for therapeutic intervention (Chen and Wang, 2012).

The conversion of androgen-dependent prostate cancer cells (ADPC) to androgen- independent prostate cancer cells (AIPC) has been shown to lead to decreased responses to hormone therapy and increased mortality rates associated with prostate cancer (Kahn *et al.*, 2014). A better understanding of the molecular mechanisms that mediate the conversion from ADPCs to AIPCs is warranted to devise effective prostate cancer suppression strategies. Recently, researchers chemically induced the conversion of LNCaP cells, an androgen-dependent prostate cancer cell line, to an androgen-independent phenotype to identify signal transduction pathways and regulatory genes involved in the transformation from an androgen-dependent to an androgen-independent phenotype using microarray analysis (Liu *et al.*, 2014). Analysis revealed 347 differentially expressed genes (156 up-regulated and 191 down-regulated). It was observed that androgen-independent transformation involves cell proliferation, differentiation, cell cycle control, and protein metabolism as key biological functions and involves the p53 signaling pathway, MAPK signal pathway, TGF-β signal pathway, and JAK-STAT signal pathway. Critical genes and signal transduction pathways identified from the study may potentially serve as molecular targets to prevent or attenuate the development of androgen-independent prostate cancer cells.

In an earlier study it was demonstrated using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) methods that overexpression of AT2R induced apoptosis in androgen-independent (DU145 and PC3) and androgen-dependent (LNCaP) cell lines (Li *et al.*, 2009). It was also shown that overexpression of AT2R in DU145 cells containing the AT2R recombinant adenovirus significantly decreased cell proliferation and significantly slowed the progression from the G1-phase of the cell cycle to the S-phase of the cell cycle. Overexpression studies like the aforementioned study are vital to cancer biology research, however, when overexpression studies are coupled with gene expression analysis a dynamic picture regarding the specific molecular mechanisms that underpin various biological effects is produced. Gene expression profiling of DU145 cells transduced

with an AT2R recombinant adenovirus revealed that several pro-apoptotic genes (Gadd45a and HRK) were up-regulated. Subsequent short-interfering RNA (siRNA) gene silencing studies designed to abolish Gadd45a and HRK protein expression reduced the apoptotic effect by 30% and 50%, respectively and provides proof-of-principle that gene expression profiling can lead to novel targets that may aid in cancer research (Pei *et al.*, 2014). The data generated from this study not only provides potential interesting target genes to treat prostate cancer but also provides the underlying molecular details for the observed biological effects in DU145 cells containing the AT2R recombinant adenovirus.

Noninvasive or *in situ* bladder cancer, the type of cancer located in the internal regions of the bladder, is the predominantly diagnosed form of bladder cancer according to the American Cancer Society (American Cancer Society [ACS], 2015). Mengual *et al.* (2009) employed DNA microarray expression profiling to determine relevant markers that could improve diagnosis and the development of diagnostic assays for bladder cancer. Differentially expressed genes were identified from tissue and urine samples from bladder cancer patients. Bioinformatics analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) validation studies revealed that both bladder cancer cells and bladder fluids from patients yield similar gene expression signatures. Results suggest the efficacy of employing gene expression profiling to identify new mRNA targets to diagnose noninvasive bladder cancer.

Advanced squamous cervical cancer, like many of the oncologic disorders discussed in this review, is a frequently diagnosed human cancer with a poor prognosis and undesirable responses to current treatment strategies attributable to intrinsic and acquired resistance (Balacescu *et al.*, 2014). Recently, a study was performed to investigate the gene expression profile associated with treatment failure due to intrinsic resistance mechanisms. In the study, advanced cervical cancer patients were divided into two groups based on their response to clinical treatment (responder and non-responder) and whole human gene expression microarray experiments were performed to identify aberrant molecular processes and signal transduction pathways associated with non-responsiveness to standard treatment strategies. Bioinformatics exploration using Ingenuity Pathway Analysis were confirmed using qRT-PCR and immunohistochemical procedures. DNA repair mechanisms and molecules such as BRCA1, BRIP1, and RAD51 were among the most statistically significant nuclear proteins in non-responding patients (Balacescu *et al.*, 2014). BRCA1 is a known tumor suppressor protein found in humans and repeatedly implicated in human carcinomas, most notably breast cancer, and is responsible for repairing double-stranded breaks in DNA (Savage and Harkin, 2015). It is therefore not surprising that dysregulation of the BRCA1 canonical pathway is involved in mediating intrinsic resistance. It is possible that BRCA1, BRIP1, and RAD51 could serve as excellent predictor molecules regarding the success rate of clinical therapy to advanced squamous cervical cancer in women.

Cell models have been used for many decades by biomedical researchers to study the effects of chemical substances on biological systems. However, the major concern with utilizing a cell model to investigate the effects of therapeutic solutions for a particular disease stems from the variability of *in vitro* cell culture techniques designed to grow eukaryotic cells. Differences in cell culture methods such as media formulations, cell passage protocols, and other treatment procedures performed in basic research laboratories could potentially contribute to observed beneficial clinical effects.

To validate prospective translational advantages of the use of human papillomavirus (HPV)-mediated carcinogenesis cell models Wan *et al.* (2008) compared previously published cervical intraepithelial neoplasia (CIN) gene expression data with new oligonucleotide microarray data from early passage and late passage HPV16-immortalized human keratinocyte (HKc) cell lines. Two genes SIX1

and GDF15 were found to be highly overexpressed in both cell model and tissue arrays of cervical cancer and thus identified as potential biomarkers. Overexpression of Six1, a transcription factor, in HPV16-immortalized human keratinocyte cell lines (HKc/HPV16) has been shown to contribute to cellular proliferation, migration, and invasion. Additionally, increased Six1 expression was also shown to serve as a key regulator in the conversion of early stage HPV16-mediated transformation to a differentiation-resistant phenotype (Xu *et al.*, 2015). Targeted molecular approaches to inhibit Six1 expression may serve as an advantageous anti-tumor strategy for many different types of human cancers according to previous research studies (Wu *et al.*, 2015). Figure 2 shows predicted and known Six1 molecular associations based on bioinformatics analysis.

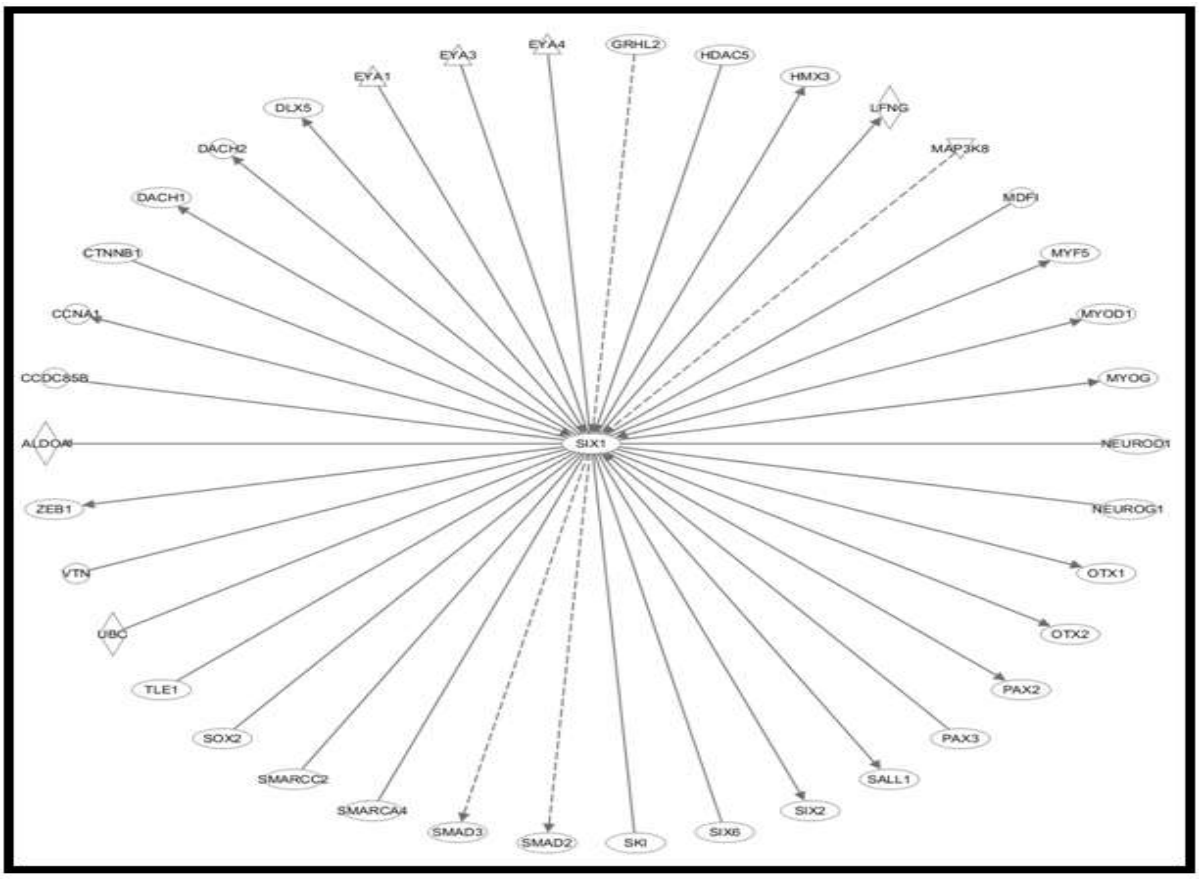

Fig. 2. Gene network for SIX1 generated using Ingenuity Pathway Analysis (IPA). Shapes denote different cellular molecules: kinase (▽), phosphatase (△), peptidase (◊), transcriptional regulator (○), and other (○). Arrows correspond to the following relationships: acts on (A➔B), direct interaction (—), and indirect interaction (----).

Bioinformatics analysis reveals that Six1 directly activates another transcription factor Zeb1, a protein that has been shown to be involved in epithelial-mesenchymal transition and metastasis in cervical cancer and colorectal cancer (Chen *et al.*, 2013; Ono *et al.*, 2012). Both Six1 and Zeb1 may serve as potentially strong therapeutic targets and biomarkers. Similar gene expression profiles from *in vitro* cell models of CIN and cervical cancer provide compelling evidence that both the model system and cervical cancer may also share homologous cellular features and therefore may respond in a similar manner in response to potential chemotherapeutic treatments.

On a global scale gastric cancer continues to contribute to a high mortality rate worldwide. Thus, there are ongoing biomedical research efforts involving microarray analysis to improve diagnostic

and prognostic indicators associated with gastric cancer. Defining specific genetic features of different stages of tumor development will lead to the ability to correlate symptomatology and tumor activity such as proliferation, differentiation, and drug-resistance with gene expression profiles and lead to the development of resolute gastric cancer treatment options (D'Angelo *et al.*, 2014).

In 2014, a microarray-based gene expression study was performed to ascertain specific transcription factors and microRNAs (miRNAs) associated with gastric cancer. Following microarray analysis 977 differentially expressed genes were identified.

Bioinformatics software implicates cell cycle metabolism as an important biological process related to gastric cancer and further identified 492 down-regulated genes and 485 up-regulated genes. Several novel microRNAs (miRNAs) and transcription factors (TFs) were specifically identified in the experiment including SPI1, THAP1, and miR-557. The identified genes may be critical to the development and progression of gastric cancer based on the understanding that their target genes have previously been implicated in gastric cancer (Jiang *et al.*, 2014). Future functional experimental analysis involving gene or protein knockdown will provide additional evidence regarding the roles microRNAs and transcription factors play in gastric cancer and may serve as meaningful molecular indicators of various tumorigenic stages and may even help predict potential therapeutic strategies for treating gastric cancer.

Limitations to Microarray Studies and Bioinformatics Analysis

While gene expression data is extremely beneficial and provides strong indicators of probable macromolecular associations, a major limitation of bioinformatics analysis and other *in silico* investigations is that algorithm-based predictions may not accurately reflect actual intracellular events and functions. Moreover, issues such as cellular heterogeneity of experimental tissues must be addressed to eliminate inaccurate gene expression measurements of specific cell populations (King and Sinha, 2001).

Also gene expression validation studies (e.g., qRT-PCR) and microscopic investigations such as immunohistochemistry, fluorescent microscopy, and other functional experiments must be performed in parallel to confirm microarray results. The above information suggests that while microarray data is an excellent starting point to investigate a wide variety of aberrant physiological activities, additional experiments must be performed to ensure that data generated from computer-assisted analysis is precise and is consistent with molecular associations and cellular function.

Conclusion

Microarray technology has the potential to play a major role in personalized medicine and other clinical applications. Analysis of an individual's gene expression profile and the establishment of an accurate determination of differentially expressed genes from different tissues could be used to enhance the diagnosis of specific diseases and offer relevant prognostic indicators to various treatments.

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