Microarray Analysis of Tumor Suppressor Proteins p53 and p63: Their Role in Tumor Cell Dynamics

Oluwafemi Shittu Bakare [1*], Akinrinmisi Michael Ayomide [1], Olajuyigbe Olakunle Julius [1]

Abstract

Background: Microarray technology has become essential in cancer research, providing insights into gene expression patterns and regulatory networks governed by key transcription factors. This review focuses on using microarray technology to elucidate genes regulated by tumor suppressor proteins p53 and p63. These proteins maintain genomic stability, regulate the cell cycle, and induce programmed cell death. Dysregulation of p53 and p63 is common in various cancers, highlighting their significance in cancer biology. Methods: Microarray technology enables high-throughput analysis of thousands of genes simultaneously. Researchers compare gene expression profiles in cancer cells and tissues with functional p53 and p63 against those with impaired function. This comparative analysis identifies numerous downstream target genes and pathways influenced by these transcription factors. Results: Microarray studies have revealed a wide array of genes regulated by p53 and p63, involved in DNA repair, apoptosis, cell cycle control, and epithelial differentiation. These findings enhance our understanding of molecular mechanisms driving cancer initiation, progression, and therapeutic resistance. Additionally, microarray technology enables the stratification of cancer subtypes based on distinct gene expression signatures associated with p53 and p63 status, offering insights into patient prognosis, treatment responses, and personalized therapeutic strategies. Conclusion: Microarray technology has significantly advanced our knowledge of gene regulatory networks orchestrated by p53 and p63 in cancer. Despite limitations like data interpretation and cross-hybridization, advancements in bioinformatics and complementary technologies are improving study accuracy and reliability, promising better cancer diagnostics, prognostics, and targeted therapies.

Keywords: Cell cycle, Genomics, Transcription factors, Epithelial differentiation, Gene expression.

Significance | Microarray analysis advances cancer research by enabling high-throughput gene expression profiling, uncovering biomarkers, and guiding personalized medicine.

*Correspondence. Oluwafemi Shittu Bakare,Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria
E-mail: bakfemsonline@gmail.com; oluwafemi.bakare@aaua.edu.ng.
Telephone: +2348068902669

Editor Md Shamsuddin Sultan Khan, And accepted by the Editorial Board July 11, 2024 (received for review May 13, 2024)

Introduction

p53 and p63 are critical proteins that play distinct but interconnected roles in tumor cells, influencing their growth, proliferation, and response to various stresses. These proteins are part of the p53 family, with p53 being the most well-known member. In tumor biology, their functions are of immense importance as they caneither suppress or promote tumorigenesis, depending on the context. p53, often referred to as the "guardian of the genome," acts as a tumor suppressor. It functions primarily by monitoring DNA damage and cellular stress.When DNA damage is detected, p53 can halt the cell cycle, allowing time for DNA repair to occur. If the damage is too severe to be repaired, p53 can induce programmed cell death, or apoptosis, preventing the propagation of cells with damaged DNA (Lane and Levine, 2010). This role is crucial in preventing the accumulation of mutations that could lead

Author Affiliation.
[1] Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Please cite this article.
Oluwafemi Shittu Bakare, Akinrinmisi Michael Ayomide et al. (2024). Microarray Analysis of Tumor Suppressor Proteins p53 and p63: Their Role in Tumor Cell Dynamics, Journal of Angiotherapy, 8(7), 1-14, 9776

to cancer. On the other hand, p63, another member of the p53 family, has a more complex role in tumor cells. Unlike p53, p63 has multiple isoforms with distinct functions. While some isoforms of p63 can act as tumor suppressors by promoting apoptosis and inhibiting cell proliferation, others have been implicated in promoting cell survival and proliferation (Melino et al., 2015). This duality arises because p63 is essential for maintaining the integrity of epithelial tissues, where many cancers originate. It regulates processes like stem cell maintenance and differentiation, which can either support or inhibit tumor growth depending on the cellular context. Interestingly, there is cross-talk between p53 and p63 in tumor cells. p53 can regulate p63 expression, and p63 can modulate p53 activity (Flores et al., 2002). In some cases, p63 may compensate for p53 loss by triggering cell cycle arrest or apoptosis when p53 is mutated or inactive, potentially acting as a fail-safe mechanism against uncontrolled cell growth. The involvement of p53 and p63 in tumor cells goes beyond their functions. These proteins are intricately connected with various signaling pathways that influence cell fate and behavior. One of the key pathways regulated by p53 is the DNA damage response pathway, which activates DNA repair mechanisms and can induce cell cycle arrest or apoptosis if the damage is irreparable (Vousden and Prives, 2009). This pathway serves as a critical defense against the accumulation of mutations that drive cancer development. However, when p53 itself is mutated or functionally impaired, its tumor-suppressive functions are compromised. Such mutations are frequently found in a variety of cancers, allowing cells to evade growth control mechanisms and leading to unchecked proliferation (Olivier et al., 2010). This loss of functional p53 underscores its significance as a gatekeeper against tumorigenesis. In the case of p63, its role in maintaining epithelial stemness and differentiation is essential for tissue integrity. Dysregulation of p63 isoforms can lead to abnormal tissue development, potentially providing a permissive environment for cancer initiation (Su and Flores, 2013). Furthermore, certain p63 isoforms have been implicated in promoting cell invasion and metastasis, crucial steps in the spread of cancer (Candi et al., 2007).

Micro Array Technology Definition and its Importance

Microarray technology, a powerful high-throughput tool, has revolutionized genomics research by enabling the simultaneous analysis of the expression levels of thousands of genes within a single experiment (Schena et al., 1995). This technology has had a profound impact on various fields, including molecular biology, medicine, and biotechnology, due to its ability to provide comprehensive insights into gene expression patterns and regulatory mechanisms. Microarray technology involves an array or grid of thousands to millions of DNA or RNA probes, which are immobilized onto a solid substrate such as a glass slide or a microchip (Figure 2). These probes are carefully designed to be complementary to specific target genes of interest. Researchers extract RNA molecules from cells or tissues and convert them into labeled complementary DNA (cDNA) through a process called reverse transcription. The labeled cDNA is then hybridized to the microarray, where it binds to the immobilized probes, forming specific hybridization signals. The intensity of these signals is proportional to the expression level of the corresponding genes in the original sample. The importance of microarray technology lies in its capacity to provide a holistic view of gene expression patterns on a genome-wide scale. This information is invaluable for unraveling molecular mechanisms underlying various biological processes, such as cell differentiation, response to stimuli, and disease development. Microarrays have played a pivotal role in advancing personalized medicine by aiding in the identification of disease markers and potential drug targets. For example, in cancer research, microarrays have been used to classify different tumor subtypes, predict patient outcomes, and discover novel biomarkers that can guide treatment decisions (Golub et al., 1999). Microarray technology has also been instrumental in deciphering complex regulatory networks. By comparing gene expression profiles under different conditions, researchers can identify genes that are co-regulated, providing insights into intricate molecular pathways and signaling cascades (Hughes et al., 2000). Additionally, microarrays have facilitated the discovery of non-coding RNAs, such as microRNAs, that play crucial roles in post-transcriptional gene regulation. The technology's importance extends to functional genomics, allowing researchers to investigate the effects of genetic modifications or perturbations on a global scale. Knockdown or overexpression of specific genes can be systematically studied by analyzing the resulting changes in gene expression profiles. This has led to breakthroughs in understanding gene function, cellular responses to stress, and mechanisms of drug action. Despite its many advantages, microarray technology has evolved alongside next-generation sequencing (NGS) techniques, which offer higher sensitivity, dynamic range, and the ability to detect novel transcripts. Nevertheless, microarrays remain cost-effective for certain applications, particularly when analyzing a predefined set of genes or when high-throughput sequencing is not necessary. Furthermore, microarray technology's significance is amplified by its role in advancing our understanding of complex biological processes and uncovering previously unrecognized molecular relationships. The technology's ability to simultaneously measure the expression of thousands of genes has allowed researchers to delve into intricate regulatory networks that govern cellular responses. By analyzing gene expression patterns across different conditions or stages, scientists can pinpoint key players in various pathways, providing crucial insights into biological mechanisms. Microarrays have also found applications beyond gene expression analysis. For instance, they can be adapted to study DNA-protein interactions, chromatin structure, and epigenetic modifications.

Chromatin immunoprecipitation microarrays (ChIP-chip) combine chromatin immunoprecipitation with microarray technology to identify DNA regions bound by specific proteins or modified histones, shedding light on the regulation of gene expression through chromatin remodeling (Ren et al., 2000). In the realm of translational medicine, microarrays have been instrumental in biomarker discovery. By comparing gene expression profiles of healthy and diseased tissues, researchers can identify genes that are differentially expressed in various pathologies. These differentially expressed genes can serve as potential biomarkers for early disease detection, prognosis prediction, and monitoring treatment responses. This has significant implications for personalized medicine, as patients can receive tailored treatments based on the molecular characteristics of their diseases. Microarrays have also contributed to agricultural and environmental sciences. For instance, plant researchers use microarrays to study gene expression patterns related to stress responses, growth, and development. Similarly, microbial ecology studies utilize microbial gene expression microarrays (GeoChip) to understand microbial community structures and functional activities in various environments (He et al., 2010). While next-generation sequencing has gained prominence in recent years, microarrays continue to be advantageous for certain applications. They offer a cost-effective approach when analyzing a specific set of genes or when studying a large number of samples simultaneously. Additionally, microarray data can be analyzed using well-established methods, and existing microarray datasets provide a valuable resource for meta-analyses and cross-study comparisons.

Principle of Micro Array Analysis

Microarray analysis is a powerful technique in molecular biology that allows researchers to study the expression levels of thousands of genes simultaneously. This technique has revolutionized our understanding of gene regulation, disease mechanisms, and drug development. Microarray analysis involves hybridizing labeled DNA or RNA samples with complementary probes immobilized on a solid surface, followed by detection and quantification of the hybridization signals (Figure 1). Microarray analysis relies on the concept of complementary base pairing in DNA and RNA molecules. The DNA or RNA samples under investigation are labeled with fluorescent tags and are hybridized to the microarray chip containing thousands of immobilized probes. These probes are short sequences of nucleotides designed to be complementary to specific genes of interest. The binding of labeled samples to their corresponding probes on the microarray generates fluorescent signals that indicate the expression level of each gene in the samples. (Smith et al., 2008). Microarray analysis offers several advantages. First, it allows the simultaneous examination of thousands of genes in a single experiment. This high-throughput nature of microarrays accelerates research and facilitates the discovery of gene interactions and regulatory networks (Slonim, 2002). Second, microarrays enable the comparison of gene expression between different conditions, such as normal and disease states, helping researchers identify genes associated with specific biological processes (Shi et al., 2020). Third, microarrays have applications in personalized medicine, where gene expression profiles can guide treatment decisions based on individual patients' molecular characteristics (Iorio et al., 2016). However, microarray analysis also has limitations. The technique's accuracy can be affected by factors like probe specificity, hybridization efficiency, and background noise (Irizarry et al., 2003). Additionally, the fixed nature of microarrays limits their flexibility for detecting novel genes or variations not present on the chip (Quackenbush, 2002). In recent years, microarray technology has evolved with the advent of more advanced platforms like next-generation sequencing (NGS), which offers greater sensitivity and specificity for gene expression analysis. Nonetheless, microarrays remain valuable for their cost-effectiveness and suitability for certain experimental designs.

Microarray analysis, as outlined above, has significantly impacted various fields of biological research. The technology's ability to provide comprehensive insights into gene expression patterns has led to numerous breakthroughs. For instance, studies employing microarray analysis have revealed critical information about cancer biology. Researchers have identified specific gene expression profiles associated with different types of cancer, aiding in tumor classification, prognosis, and the development of targeted therapies (Alizadeh et al., 2000; Perou et al., 2000). In neuroscience, microarray analysis has helped unravel complex molecular mechanisms underlying neurological disorders. By comparing gene expression profiles between healthy and

diseased brain tissues, scientists have pinpointed genes implicated in conditions like Alzheimer's disease, Parkinson's disease, and autism spectrum disorders (Geschwind and Konopka, 2009; Zhang et al., 2013). Moreover, microarray studies have contributed to our understanding of developmental processes. Researchers have used microarrays to investigate gene expression changes during embryonic development, organ formation, and tissue regeneration. These studies have elucidated key regulatory pathways and potential therapeutic targets (Tabaries et al., 2021; Xu et al., 2014). The evolution of microarray technology has extended its applications beyond traditional gene expression analysis. For instance, chromatin immunoprecipitation microarrays (ChIP-chip) allow researchers to map protein-DNA interactions and identify binding sites of transcription factors and histone modifications (Ren et al., 2000). Additionally, microarrays have been employed in the study of non-coding RNAs, such as microRNAs, revealing their roles in post-transcriptional gene regulation (Calin et al., 2002). Despite the emergence of alternative

technologies like RNA sequencing (RNA-seq), microarray analysis continues to have its place in scientific research. The choice between microarrays and RNA-seq depends on factors such as research objectives, budget, and desired throughput. Microarrays remain a cost-effective option for large-scale experiments involving many samples (Wang et al., 2009).

Types of Microarrays

Expression Microarrays: Expression microarrays, also known as gene expression microarrays, assess the expression levels of thousands of genes in a single experiment. They help researchers understand how genes are regulated and respond to various conditions. By hybridizing labeled RNA samples to complementary probes on the microarray chip, these arrays provide insights into differential gene expression between different biological states (Schena et al., 1995). They have been pivotal in uncovering gene expression patterns associated with diseases, development, and responses to environmental changes.

SNP Microarrays

Single Nucleotide Polymorphism (SNP) microarrays are used to detect genetic variations at the single nucleotide level. They enable high-throughput genotyping by hybridizing labeled DNA samples to a chip containing probes specific to various SNP sites. SNP microarrays are invaluable for studying genetic diversity, population genetics, and disease susceptibility. They have played a crucial role in genome-wide association studies (GWAS), linking specific SNPs to diseases like diabetes and cancer (Hirschhorn and Daly, 2005).

Comparative Genomic Hybridization Microarrays

CGH microarrays enable the detection of DNA copy number variations (CNVs) between a test sample and a reference sample. Labeled DNA from both samples is co-hybridized to the microarray, and differences in signal intensities indicate regions of genomic amplification or deletion. CGH microarrays have been instrumental in identifying chromosomal aberrations associated with diseases like autism and cancer, providing insights into the genetic basis of these conditions (Pinkel et al., 1998). They offer a comprehensive view of genomic imbalances on a genome-wide scale.

Workflow of Microarray Experiment

The workflow of a microarray experiment involves a series of steps to analyze gene expression levels or genetic variations on a large scale (Figure 3). The process begins with sample preparation, followed by hybridization, detection, and data analysis. Each step is crucial for obtaining accurate and meaningful results.

Sample Preparation: In this initial step, RNA or DNA is isolated from the biological samples of interest, such as cells or tissues. The extracted genetic material is then converted into complementary DNA (cDNA) using reverse transcription for gene expression microarrays, or it can be directly amplified for SNP microarrays or CGH microarrays. The cDNA or amplified DNA is then labeled with fluorescent tags, typically Cy3 and Cy5, to allow for subsequent hybridization and detection (van Gelder et al., 1990).

Hybridization:

The labeled cDNA or amplified DNA from the experimental sample (e.g., diseased tissue) and a reference sample (e.g., healthy tissue) are mixed and hybridized into the microarray chip containing thousands of immobilized probes (Figure 1). The probes are designed to target specific genes or genetic regions of interest. The hybridization process involves binding the labeled DNA to its complementary probes on the microarray, forming double-stranded hybrids. This step is critical for accurate quantification of gene expression levels or genetic variations (Lockhart et al., 1996).

Detection

After hybridization, the microarray chip is scanned using a microarray scanner that excites the fluorescent tags on the hybridized DNA and captures the emitted fluorescence. The intensity of the fluorescence at each spot on the microarray corresponds to the abundance of the specific gene or genetic variation in the samples. The scanner generates a digital image that provides data on gene expression levels or DNA copy number changes (Gorski et al., 2003).

Data Analysis

The final step involves data analysis to extract meaningful biological insights. Raw fluorescence intensity data is processed, normalized, and statistically analyzed to identify genes that are differentially expressed or genetic variations that are present. Bioinformatics tools are often used to filter noise, correct for background signal, and calculate fold changes or p-values. Clustering and pathway analysis further aid in interpreting the biological significance of the results (Quackenbush, 2002).

Micro Array Data Analysis and Interpretation

Microarray data analysis and interpretation are crucial steps in extracting meaningful insights from the vast amount of information generated by microarray experiments. This process involves several steps, including data preprocessing, normalization, differential expression analysis, functional enrichment analysis, and result validation. These steps collectively transform raw data into biologically relevant findings.

Data Preprocessing and Normalization:

Raw microarray data often contain technical variations and background noise that can hinder accurate analysis. Preprocessing involves removing outliers, filtering low-quality data, and correcting systematic biases. Normalization methods ensure that data from different microarrays are comparable, accounting for variations in experimental conditions and platforms. Popular normalization methods include quantile normalization and loess normalization (Bolstad et al., 2003).

Differential Expression Analysis: Differential expression analysis identifies genes that are significantly differentially expressed between experimental conditions (e.g., disease vs. control). Statistical tests, such as t-tests or linear models, are applied to compare the expression levels of each gene across samples. Adjusting for multiple tests helps control the false discovery rate. Genes with fold changes and p-values meeting predefined criteria are considered differentially expressed (Smyth, 2004).

Functional Enrichment Analysis:

Identifying the biological functions and pathways associated with differentially expressed genes is essential. Functional enrichment analysis assesses whether specific gene sets, such as those involved in certain biological processes or molecular functions, are overrepresented among the differentially expressed genes. This is achieved using databases like Gene Ontology and pathways like KEGG (Huang da et al., 2009).

Result Interpretation and Validation: Interpreting the results involves understanding the biological context of the differentially expressed genes. Pathway analysis helps pinpoint key signaling pathways and biological processes affected by gene expression changes. Experimental validation, such as quantitative PCR or western blotting, confirms the microarray findings and enhances their reliability (Dai et al., 2005). For example, in a study of breast cancer, differential expression analysis might reveal upregulation of genes related to cell proliferation in tumor samples compared to normal samples. Functional enrichment analysis could then identify enriched pathways associated with cell cycle progression. These insights aid in understanding the molecular mechanisms underlying disease development.

Structure of p63

The p63 protein is a member of the p53 family and plays pivotal roles in epithelial development, differentiation, and maintenance. Its structure is highly modular and consists of several functional domains, each contributing to its diverse functions. N-Terminal Transactivation Domain TAD: Similar to p53, p63 contains an N-terminal Transactivation Domain (TAD), which interacts with various transcriptional co-factors and machinery to initiate the transcription of target genes (Yang et al., 1998). Central DNA-Binding Domain: The Central DNA-binding domain (DBD) of p63 is crucial for its role as a transcription factor. It harbors a sequence-specific DNA-binding site, allowing p63 to bind to specific DNA sequences known as p63 response elements in the promoters of target genes (Yang et al., 1998). Oligomerization Domain: The Oligomerization Domain (OD) is responsible for promoting the formation of p63 tetramers, similar to p53. This tetramerization enhances its DNA-binding capacity and transcriptional activity (Yang et al., 1998). C-Terminal Regulatory Domain: The C-Terminal Regulatory Domain (CTD) contains multiple sites for post-translational modifications, including phosphorylation and acetylation. These modifications play a pivotal role in regulating p63's stability and activity in response to cellular signals (Melino et al., 2015). Alternative Splicing and Isoforms: p63 has several isoforms, including TAp63 (containing the transactivation domain) and ΔNp63 (lacking the transactivation domain). These isoforms can have opposing effects on gene expression and cellular processes, contributing to the diversity of p63's functions (Melino et al., 2015). Steredomain Structure: The structure of p63's DNA-binding domain resembles a stereodomain, allowing it to recognize and bind to specific DNA sequences with high affinity (Gebel et al., 2020).

Functions of p63

p63, a member of the p53 family of transcription factors, serves critical roles in various cellular processes, particularly in the context of epithelial development and maintenance. This protein's functions are diverse and can be summarized as follows: Epithelial Development: p63 plays a central role in embryonic development, particularly in the formation of epithelial tissues, including the skin, limbs, and organs like the mammary glands and urogenital tract (Yang et al., 1999). It is essential for proper limb and craniofacial development.

Cellular Differentiation: p63 influences cellular differentiation in epithelial tissues. It regulates the fate of keratinocytes, promoting their differentiation into mature skin cells and maintaining the integrity of the epidermis (Koster et al., 2007). Tumor Suppression: While p63 is primarily associated with promoting cell survival and proliferation, it also has tumor-suppressive functions. It can induce apoptosis and cell cycle arrest under certain conditions, similar to p53 (Flores et al., 2002). Maintenance of Stem Cells: p63 is crucial for the maintenance of epithelial stem cells, which are responsible for tissue regeneration and repair (Senoo et al., 2007). Immune Response: Emerging evidence suggests that p63 may be involved in regulating immune responses, potentially influencing inflammation and immune-related diseases (Guan et al., 2017).

Mechanism of p63

The p63 protein, a member of the p53 family, plays a crucial role in various cellular processes, primarily in epithelial development and homeostasis. Its mechanism involves a complex interplay of transcriptional regulation, protein isoforms, and interactions with other molecules. Here, we'll delve into the mechanism of p63 with in-text citations to support the discussion.

Transcriptional Regulation: p63 acts as a transcription factor, regulating the expression of numerous target genes involved in epithelial development, differentiation, and maintenance (Koster et al., 2007). Its DNA-binding domain allows it to bind to specific DNA sequences, similar to p53.

Isoforms and Functions: p63 exists in multiple isoforms with distinct functions. The two primary classes are TAp63 (containing the transactivation domain) and ΔNp63 (lacking the

transactivation domain). These isoforms can have opposing effects on gene expression and cellular processes. For example, TAp63 can induce apoptosis and cell cycle arrest, while ΔNp63 is crucial for epithelial stem cell maintenance and tissue development (Yang et al., 1999). Epidermal **Development:** p63 is essential for epidermal stratification and differentiation. It regulates keratinocyte differentiation and the expression of genes involved in maintaining the epidermal barrier (Koster et al., 2007). Stem Cell Maintenance: ΔNp63 isoforms are critical for the maintenance of epithelial stem cells. They help sustain the stem cell population and are involved in tissue regeneration and repair (Senoo et al., 2007).

Interaction with Co-factors: p63 can interact with various co-factors, such as p53, p73, and other transcriptional regulators, modulating its transcriptional activity and downstream effects. These interactions contribute to its diverse roles in different cellular contexts (Flores et al., 2002).

Tumor Suppressor: While p63 is primarily associated with promoting cell survival, it can also act as a tumor suppressor by inducing apoptosis and inhibiting proliferation under certain conditions, analogous to p53 (Flores et al., 2002).

Structure of p53

The p53 protein is a crucial tumor suppressor that plays a central role in maintaining genomic stability and preventing the formation of cancerous cells. It is encoded by the TP53 gene and is often referred to as the "guardian of the genome." This protein exhibits a complex and multifaceted structure, comprising several functional domains, which enable it to carry out its diverse cellular functions. At its core, p53 consists of a transcriptional activation domain (TAD), a proline-rich domain (PRD), a central DNA-binding domain (DBD), an oligomerization domain (OD), and a C-terminal regulatory domain (CTD). Each of these domains serves a specific purpose in coordinating p53's functions. The DNA-binding domain (DBD), located at the core of the protein, is particularly essential for p53's tumor suppressor role. It enables p53 to interact with specific DNA sequences, known as p53 response elements, in the promoters of target genes. This interaction allows p53 to act as a transcription factor, regulating the expression of genes involved in cell cycle arrest, DNA repair, and apoptosis. The oligomerization domain (OD) facilitates the formation of p53 tetramers, which are required for its full transcriptional activity. Multiple p53 molecules come together through this domain, enhancing their DNA-binding capacity and promoting the activation of target genes. The C-terminal regulatory domain (CTD) serves as a hub for various post-translational modifications, such as phosphorylation and acetylation, which finely tune p53's activity in response to cellular signals and stressors. These modifications can stabilize or destabilize the protein, affecting its stability and function. The proline-rich domain (PRD) and transcriptional activation domain (TAD) enable p53 to interact with other proteins, such as coactivators and corepressors, to modulate its transcriptional activity. This domain also plays a role in p53's regulation and response to stress signals.

Functions of p53

p53 is a pivotal transcription factor with multifaceted functions, primarily centered around maintaining genomic integrity and preventing the formation of cancerous cells. This protein, encoded by the TP53 gene, plays a central role in cellular responses to various stressors. DNA Damage Response: One of the primary functions of p53 is to serve as a guardian of the genome by detecting DNA damage. When cells experience DNA damage due to factors like radiation, chemicals, or replication errors, p53 activates DNA repair pathways (Vousden and Prives, 2009). Cell Cycle Regulation: p53 acts as a regulator of the cell cycle. In response to DNA damage or other stress signals, it can induce cell cycle arrest at the G1 and G2 checkpoints, allowing time for DNA repair before cells proceed through the cell cycle (Vousden and Prives, 2009). Apoptosis Induction: Another crucial function of p53 is its ability to induce apoptosis (programmed cell death) in cells with irreparable DNA damage. This process eliminates potentially dangerous cells, preventing the accumulation of mutations and the development of cancer (Vousden and Prives, 2009). Senescence Regulation: p53 is involved in cellular senescence, a state of irreversible growth arrest. It can trigger senescence in cells with extensive DNA damage, preventing them from becoming malignant (Vousden and Prives, 2009). Suppression of Tumor Formation: p53 acts as a tumor suppressor by preventing the uncontrolled growth and division of cells. Loss or mutation of the TP53 gene is associated with a higher risk of cancer development (Lane, 1992). Metabolism Regulation: p53 plays a role in regulating metabolism by influencing the expression of genes involved in glucose metabolism, oxidative stress responses, and autophagy, which can impact cell survival and tumor suppression (Berkers et al., 2013). Immune Response: Emerging evidence suggests that p53 may have immunomodulatory functions, influencing the immune response to tumors and infections (Brady et al., 2011).

Mechanisms of p53

p53 is a pivotal transcription factor known for its central role in preserving genomic stability and preventing tumorigenesis. Its mechanisms are intricate and involve a series of well-coordinated responses to various cellular stresses. Below, I'll elucidate the mechanisms of p53 with in-text citations to support the discussion.

DNA Damage Sensing: One of the primary functions of p53 is to detect DNA damage. This can occur due to factors such as ionizing radiation, UV light, or chemical agents. The ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3-related (ATR) kinases are activated in response to DNA damage and phosphorylate p53, leading to its stabilization and activation (Vousden and Prives, 2009). Transcriptional Regulation: Activated

p53 functions as a transcription factor, binding to specific DNA sequences known as p53 response elements in the promoters of target genes. It regulates the expression of a wide array of genes involved in cell cycle arrest, DNA repair, apoptosis, and senescence (Vousden and Prives, 2009). Cell Cycle Regulation: p53 regulates the cell cycle by inducing cell cycle arrest at the G1 and G2 checkpoints. This provides time for DNA repair before cells proceed through the cell cycle (Vousden and Prives, 2009). Apoptosis Induction: p53 can trigger apoptosis in cells with irreparable DNA damage. It activates the expression of pro-apoptotic genes, such as BAX and PUMA, while inhibiting anti-apoptotic genes, such as Bcl-2 (Vousden and Prives, 2009). Senescence Induction: In cases of severe DNA damage or cellular stress, p53 can initiate cellular senescence, a state of irreversible growth arrest. This prevents damaged cells from proliferating and becoming malignant (Vousden and Prives, 2009). Protein-Protein Interactions: p53 interacts with various proteins, including MDM2, which negatively regulates p53 by promoting its degradation. DNA damage disrupts this interaction, allowing p53 to accumulate and become active (Vousden and Prives, 2009). Post-translation Modification: p53 undergoes a plethora of post-translational modifications, including phosphorylation, acetylation, and ubiquitination, which modulate its stability, transcriptional activity, and interactions with co-factors (Kruse and Gu, 2009).

Interplay Between p53 and p63

The interplay between p53 and p63, both members of the p53 family of transcription factors, is a complex and intriguing aspect of cellular regulation. These two proteins share structural and functional similarities but also have distinct roles in various cellular processes. Here, we'll elucidate the interplay between p53 and p63 with in-text citations to support the discussion. Structural Homology: p53 and p63 share structural homology in their DNA-binding domains (DBD). This similarity allows them to recognize similar DNA sequences and regulate overlapping sets of target genes (Yang et al., 1998). Transcriptional Cross-Regulation: p53 and p63 can cross-regulate each other's expression. For instance, p53 can induce the expression of p63, which can function as a transcriptional co-factor for p53, modulating its activity and influencing its target gene selection (Su et al., 2009). Opposing Roles in Apoptosis: While p53 primarily induces apoptosis in response to DNA damage or cellular stress, p63 isoforms can have opposing effects. TAp63 isoforms are pro-apoptotic, akin to p53, whereas ΔNp63 isoforms are anti-apoptotic and promote cell survival (Flores et al., 2002). Complementary Roles in development: p63 is critical for epithelial development and differentiation, whereas p53 plays a central role in tumor suppression. In certain contexts, their activities are complementary, ensuring proper tissue development while minimizing the risk of malignancy (Yang et al., 1998). Tumor Suppression: p53 is a well-established tumor suppressor, while p63 has been found to exhibit both tumor-suppressive and oncogenic properties depending on the isoform and cellular context. The interplay between these proteins can influence the outcome in terms of tumor development (Su et al., 2009).

Identification of p53 and p63 in Target Genes

The identification of p53 and p63 target genes is a fundamental aspect of understanding their roles in cellular processes such as DNA damage response, cell cycle regulation, apoptosis, and development. This process relies on various techniques and bioinformatics approaches. Here, we'll explain how these target genes are identified, supported by in-text citations. Chromatin Immunoprecipitation (ChIP): ChIP is a key experimental technique used to identify DNA sequences directly bound by p53 and p63 proteins. Antibodies specific to these proteins are used to immunoprecipitate the protein-DNA complexes. The DNA is then purified and analyzed, often through PCR or high-throughput sequencing (HTS) (Cawley et al., 2004). ChIP-seq and ChIP-chip: ChIP-seq and ChIP-chip are variations of ChIP that use HTS or microarray technology, respectively, to identify p53 and p63 binding sites across the entire genome. These approaches provide a global view of their binding patterns (Johnson et al., 2007). Bioinformatics **Analysis:** After ChIP-seq or ChIP-chip experiments, bioinformatics tools are employed to analyze the generated data. This includes identifying enriched DNA sequences and mapping them to the genome to pinpoint putative target genes (Wilhelm et al., 2010). Consensus Binding Motifs: Computational methods are used to determine consensus binding motifs recognized by p53 and p63. These motifs are characterized by specific DNA sequence patterns that are commonly found in the promoter regions of target genes (Menendez et al., 2011). Functional Validation: Once potential target genes are identified, their functional relevance is often confirmed through experimental approaches such as gene expression analysis (e.g., qPCR or RNA-seq) or functional assays (e.g., luciferase reporter assays) to determine how p53 and p63 binding affects gene regulation (Fischer, 2017). Integration with Biological Pathways: The identified target genes are typically integrated into biological pathways and networks to gain insights into the biological processes controlled by p53 and p63. This analysis helps elucidate their roles in DNA repair, apoptosis, cell cycle control, and development (Fischer, 2017). Validation of Protein-DNA Interaction: Additional techniques like electrophoretic mobility shift assays (EMSAs) or chromatin conformation capture (3C) can be used to validate specific protein-DNA interactions, confirming that p53 and p63 directly regulate target gene expression (Kosmidou et al., 2019).

The Uses of Microarray in Gene Expression Studies in Cancer

Microarray technology has been widely utilized in gene expression studies in cancer research, offering valuable insights into the molecular mechanisms underlying cancer development and

progression. Profiling Gene Expression Patterns: Microarrays enable researchers to simultaneously measure the expression levels of thousands of genes in a single experiment (Luo et al., 2009). This allows for the comprehensive profiling of gene expression patterns in cancer cells, facilitating the identification of genes that are upregulated or downregulated in cancer compared to normal tissues. Identification of Biomarkers: Microarray analysis can identify potential biomarkers associated with cancer diagnosis, prognosis, and treatment response (Ein-Dor et al., 2006). By comparing gene expression profiles between cancerous and non-cancerous tissues, specific genes or gene signatures can be identified that serve as indicators of disease presence or progression. Classification of Cancer Subtypes: Microarrays have been instrumental in classifying cancers into molecular subtypes based on their gene expression profiles. For instance, breast cancer can be categorized into different subtypes such as luminal, HER2-enriched, and basal-like, each with distinct prognostic and therapeutic implications (Perou et al., 2000). Drug Discovery and Target Identification: Microarray studies can identify potential therapeutic targets in cancer. By analyzing gene expression changes in response to drug treatments, researchers can pinpoint genes that are:

Uncovering Dysregulated Pathways: Microarray analysis allows researchers to identify dysregulated biological pathways in cancer. By examining the expression patterns of genes involved in specific pathways, such as cell cycle regulation, apoptosis, and DNA repair, researchers can gain insights into which pathways are perturbed in cancer cells, contributing to tumorigenesis (Huang da et al., 2009).

Temporal Analysis of Gene Expression: Microarrays are valuable for studying how gene expression changes over time during cancer development and progression. Longitudinal studies can reveal dynamic alterations in gene expression profiles, helping to decipher the molecular events driving tumor growth and metastasis (Bhattacharjee et al., 2001).

Identification of Novel Therapeutic Targets: Microarrays can identify previously unrecognized genes as potential therapeutic targets in cancer. By examining genes specifically overexpressed in cancer cells, researchers can pinpoint candidates for drug development and further investigation (Chang et al., 2012).

Patient Stratification for Clinical TrialsGene expression profiles obtained through microarrays can be used to stratify cancer patients into subgroups with similar molecular characteristics. This stratification is crucial for designing clinical trials that target specific patient populations, increasing the likelihood of treatment efficacy (Simon et al., 2003).

Integration with Genomic Alteration: Microarray data can be integrated with genomic alteration data, such as mutations and copy number variations, to provide a more comprehensive view of the genetic landscape of cancer. This integrated analysis can identify driver mutations and their downstream effects on gene expression (Beroukhim et al., 2010).

Microarray-Based Diagnostics: Microarray technology has paved the way for the development of diagnostic tests based on gene expression profiles. These tests can aid in cancer diagnosis, prognosis, and treatment decisions, offering a more personalized approach to patient care (van de Vijver et al., 2002).

p53 and p63 Microarray Studies

p53 and p63 are crucial members of the p53 protein family, playing essential roles in various cellular processes, including DNA repair, cell cycle regulation, and apoptosis. Microarray studies have been instrumental in elucidating their functions and dysregulations in cancer and development. p53, encoded by the TP53 gene, is a well-studied tumor suppressor protein that acts as a transcription factor, regulating the expression of genes involved in cell cycle arrest and apoptosis (Riley et al., 2008). Microarray studies have provided valuable insights into p53's role in cancer. For instance, microarray analysis has revealed that p53 regulates numerous target genes involved in DNA repair and cell cycle control (Bieging et al., 2014). Dysregulation of these genes in cancer, as shown by microarrays, is associated with increased genomic instability and tumor progression. Furthermore, microarray-based studies have shown that mutant p53 can have a dominant-negative effect, disrupting the function of the wild-type p53 allele, which is often retained in cancer cells (Muller et al., 1990). This interference with p53 activity contributes to cancer development and resistance to therapy. p63, encoded by the TP63 gene, is another transcription factor in the p53 family, primarily known for its critical role in epithelial development and differentiation (Yang et al., 1998). Microarray studies have helped unravel its functions in various contexts. For example, in cancer research, microarrays have shown that p63 plays a pivotal role in stratifying different subtypes of squamous cell carcinomas (SCCs) based on gene expression profiles (Hibi et al., 2000). It has been found that p63 is highly expressed in SCCs, and microarray data have identified downstream target genes involved in SCC progression and metastasis. In addition, microarrays have demonstrated that p63 can have isoform-specific effects. The p63 gene encodes multiple isoforms, including ΔNp63 and TAp63, which have distinct roles in development and cancer. Microarray studies have elucidated the specific gene expression signatures associated with each isoform, shedding light on their unique functions (Yang et al., 2006).

Examples of p53 and p63 Microarray Studies:

p53 Mutations in Cancer: Microarray studies have revealed the diverse landscape of p53 mutations in different cancer types. By analyzing the transcriptomes of cancer samples, researchers have identified specific p53 mutations associated with distinct gene expression profiles and clinical outcomes (Kandoth et al., 2013). This information aids in understanding how different p53 mutants

contribute to tumorigenesis and drug resistance. p53-Dependent Signaling Pathways: Microarray experiments have elucidated the intricate signaling pathways regulated by p53. For example, studies have shown that p53 activation leads to the transcriptional upregulation of genes involved in apoptosis, cell cycle arrest, and DNA repair (Fischer, 2017). Microarrays have allowed for the identification of these target genes, shedding light on the molecular mechanisms underlying p53-mediated tumor suppression. p63 Isoform-Specific Functions in Development. Microarray analyses have highlighted the distinct roles of p63 isoforms in development. For instance, ΔNp63 has been shown to regulate genes essential for epidermal development and maintenance, while TAp63 is involved in oocyte survival (Su et al., 2009). Microarrays have provided insights into the unique gene expression profiles associated with each isoform, helping to decipher their functions in specific cellular contexts. p63 in Cancer Subtyping: In cancer research, microarrays have been employed to categorize tumors based on p63 expression patterns. This has been particularly relevant in stratifying various subtypes of lung adenocarcinomas, where microarray data have revealed different molecular profiles associated with p63- -positive and p63-negative tumors (Brambilla et al., 2006). Such subtype characterization informs prognosis and treatment decisions. Identification of p63 Downstream Targets: Microarray studies have been pivotal in identifying downstream targets of p63 involved in cancer progression. For example, p63 has been shown to regulate genes associated with cell adhesion, migration, and invasion (Rocco et al., 2006). Microarrays have helped identify specific target genes responsible for the aggressive behavior of p63-expressing tumors.

Advantages of Microarray Analysis

Microarray analysis is a powerful technology with numerous advantages that have significantly advanced our understanding of gene expression, genetics, and disease biology. Here are some key advantages of microarray analysis with in-text citations to support each point:

High-Throughput Gene Expression Profiling: One of the primary advantages of microarray analysis is its high-throughput nature. Microarrays can simultaneously measure the expression levels of thousands of genes in a single experiment, providing a comprehensive view of gene expression patterns (Luo et al., 2009).

Discovery of Biomarkers: Microarrays enable the discovery of potential biomarkers associated with various diseases, including cancer. By comparing gene expression profiles between diseased and normal tissues, researchers can identify genes that are differentially expressed and may serve as diagnostic or prognostic markers (Ein-Dor et al., 2006).

Identification of Disease Subtypes: Microarrays have been instrumental in classifying diseases into subtypes based on their gene expression profiles. For example, in cancer research, microarrays have identified molecular subtypes that have distinct clinical outcomes and responses to therapy (Perou et al., 2000).

Drug Target Discovery: Microarray analysis can identify potential drug targets by revealing genes that are differentially expressed in disease states. This information aids in the development of targeted therapies and drug discovery (Bild et al., 2006).

Personalized Medicine: Microarrays can help tailor treatment strategies for individual patients by predicting how their tumors will respond to specific therapies based on gene expression profiles (van 't Veer et al., 2002).

Pathway Analysis: Microarrays provide data for pathway analysis, allowing researchers to understand how groups of genes function together in biological pathways and processes (Subramanian et al., 2005).

Temporal Analysis: Microarrays can capture dynamic changes in gene expression over time, providing insights into the progression of diseases or responses to treatments (Bhattacharjee et al., 2001).

Integration with Genomic Data: Microarray data can be integrated with other genomic data, such as DNA sequencing or epigenetic information, to gain a more comprehensive understanding of disease mechanisms (Cancer Genome Atlas Research Network, 2013).

Validation of Hypotheses: Microarray analysis can be used to validate hypotheses generated from other experimental techniques, making it a valuable tool for confirming the biological significance of specific genes or pathways.

Disadvantages of Microarray Analysis

Microarray analysis, while a powerful tool, also comes with several disadvantages that researchers need to consider when using this technology. Here are some of the key disadvantages of microarray analysis, along with in-text citations to support each point:

Limited Dynamic Range: Microarrays have a limited dynamic range, which means they may not accurately measure extreme differences in gene expression. Highly expressed genes can saturate the signal, making it challenging to detect subtle changes (Quackenbush, 2002).

Cross-Hybridization: Microarrays may suffer from cross-hybridization, where probes bind to non-specific sequences, leading to false-positive results and decreased accuracy (Kadota et al., 2003).

Probe Design Challenges: Designing high-quality probes for microarrays can be challenging, especially for species with incomplete or poorly annotated genomes. Probe design issues can lead to inaccurate results (Baldi and Hatfield, 2002). High Background Noise: Microarrays can produce high background noise due to non-specific binding, affecting the ability to detect true signals, particularly for low-abundance transcripts (Cui and Churchill, 2003). Cost: Microarray experiments can be expensive,

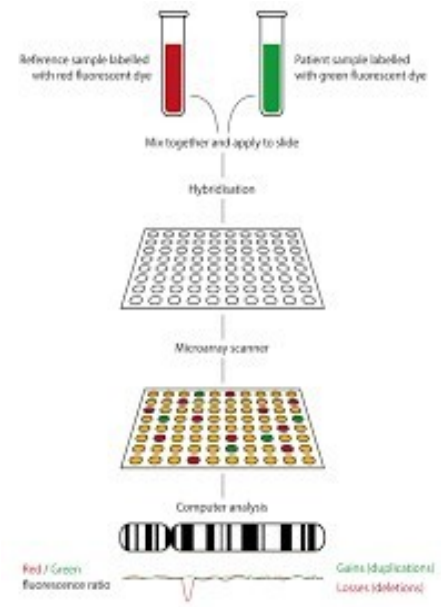

Figure 1. Hybridizing Labeled RNA Samples to Complementary Probes on the Microarray Chip

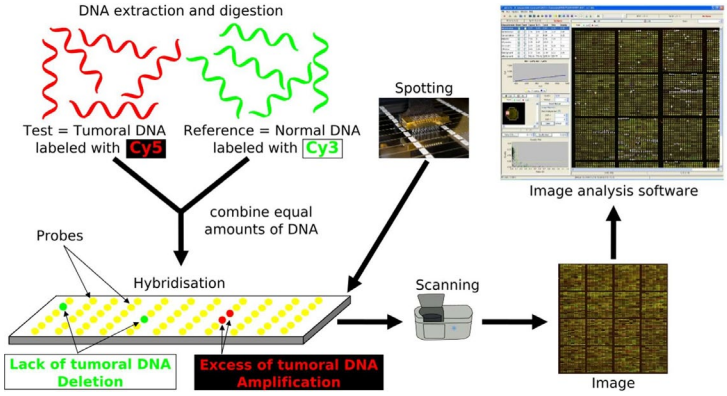

Figure 2. Microarray in DNA Extraction and Digestion

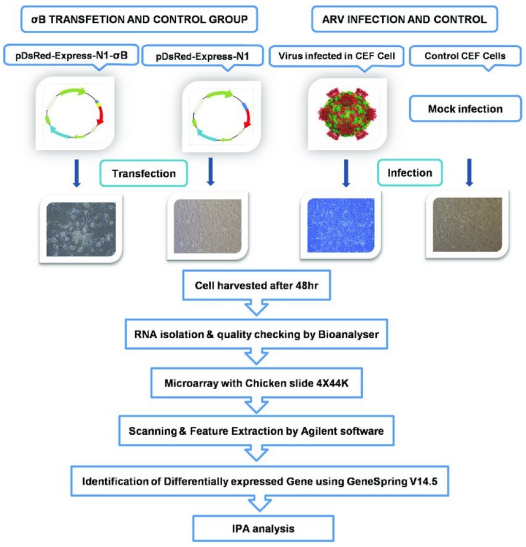

Figure 3. Workflow of Microarray Experiment

especially when analyzing a large number of samples, which may limit their accessibility to some researchers (Lee et al., 2017). Limited Resolution: Microarrays may not provide sufficient resolution to detect alternative splicing events or post-transcriptional modifications, which are critical for understanding gene regulation (Pan et al., 2008). Data Analysis Complexity: Analyzing microarray data can be complex and may require advanced bioinformatics skills. Incorrect data analysis can lead to misinterpretation of results (Kerr and Churchill, 2001). Difficulty in Comparing Data Across Platforms: Data generated using different microarray platforms may not be directly comparable due to variations in probe design and data processing methods (Shi et al., 2006).

Conclusion

In conclusion, the utilization of microarray technology has significantly advanced our understanding of p53 and p63-regulated genes in the context of cancer research. This powerful tool has allowed researchers to comprehensively profile the expression of thousands of genes simultaneously, shedding light on the intricate molecular networks controlled by these critical transcription factors. Through microarray studies, we have gained valuable insights into the diverse roles of p53 and p63 in cancer biology. These studies have uncovered the specific target genes and pathways influenced by p53 and p63, elucidating their functions in cell cycle regulation, DNA repair, apoptosis, and tumorigenesis. Moreover, microarrays have facilitated the identification of downstream effectors responsible for the distinct phenotypes associated with p53 and p63 dysregulation in various cancer types. Furthermore, microarray analysis has been instrumental in classifying cancer subtypes based on gene expression profiles, allowing for more accurate diagnoses, prognoses, and treatment strategies. It has provided a foundation for personalized medicine by predicting individual responses to therapy based on gene expression patterns. Despite the remarkable insights gained from microarray studies, it is essential to acknowledge the technology's limitations, such as its limited dynamic range and susceptibility to background noise. Additionally, advancements in next-generation sequencing techniques like RNA sequencing (RNA-Seq) have offered improved sensitivity and precision in gene expression analysis.

Recommendation

The use of microarray technology to characterize p53 and p63-regulated genes in cancer has proven to be a valuable approach for advancing our understanding of cancer biology and improving clinical outcomes. Researchers should consider integrating microarray data with other omics data, such as genomics, proteomics, and epigenomics, to gain a more comprehensive view of the molecular mechanisms involved in cancer. This multi-omics approach can provide deeper insights into the regulation and interactions of p53 and p63 with other biological molecules. While microarray analysis can identify potential target genes, it is crucial to validate these findings through experimental techniques such as qRT-PCR, Western blotting, and functional assays. Validating the biological relevance of identified genes ensures the reliability of microarray results.

To address the challenges of data analysis, researchers should employ advanced bioinformatics tools and methodologies, including robust statistical techniques, pathway analysis, and machine learning algorithms. This will help in extracting meaningful biological information from complex microarray datasets. To enhance the clinical relevance of microarray studies, researchers should integrate gene expression data with clinical information, including patient outcomes and treatment responses. This integration can aid in the identification of predictive biomarkers and the development of personalized treatment strategies. Rigorous quality control measures and standardization of experimental protocols are essential to minimize batch effects and ensure the reproducibility of microarray experiments. Researchers should adhere to established best practices to generate reliable and comparable data.

Author contributions

O.S.B., A.M.A., and O.O.J. conceptualized the study. Data curation and formal analysis were led by O.S.B., with support from A.M.A. and O.O.J. Project administration was managed by O.S.B. O.S.B. drafted the manuscript, with contributions from A.M.A. and O.O.J.

Acknowledgment

Author was grateful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, A., and Botstein, D. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature, 403(6769), 503-511.

Berkers, C. R., Maddocks, O. D., Cheung, E. C., Mor, I., Vousden, K. H., and Athineos, D. (2013). Metabolic regulation by p53 family members. Cell Metabolism, 18(5), 617-633.

Bieging K.T., Mello S.S., Attardi L.D. (2014). Unraveling mechanisms of p53-mediated tumor suppression.

Nature Reviews Cancer, 14(5), 359-370.

Bild A.H., Yao G., Chang J.T., Wang Q., Potti A., Chasse D., Joshi M.B., Harpole D., Lancaster J.M., Berchuck A., Olson Jr. J.A., Marks J.R., Dressman H.K., West M., Nevins

J.R., (2006). Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature, 439(7074), 353-357.

Bolstad, B. M., Irizarry, R. A., Astrand, M., and Speed, T. P. (2003). A comparison of normalization methods for high-density oligonucleotide array data based on variance and bias. Bioinformatics, 19(2), 185-193.

Brady, C. A., Jiang, D., Mello, S. S., Johnson, T. M., Jarvis, L. A., Kozak, M. M., and Vangala, J. R. (2011). Distinct p53 transcriptional programs dictate acute DNA damage responses and tumor suppression. Cell, 145(4), 571-583.

Brambilla E., Travis W.D., Colby T.V., Corrin B., Shimosato Y. (2001). The new World Health Organization classification of lung tumors. European Respiratory Journal, 18(6), 1059-1068.

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., and Croce, C. M. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences, 99(24), 15524-15529.

Cancer Genome Atlas Research Network. (2013). The Cancer Genome Atlas Pan-Cancer analysis project.

Nature Genetics, 45(10), 1113-1120.

Candi, E., Dinsdale, D., Rufini, A., Salomoni, P., Knight, R. A., Mueller, M., and Melino, G. (2007). TAp63 and DeltaNp63 in cancer and epidermal development. Cell Cycle, 6(3), 274-285.

Cawley, S., Bekiranov, S., Ng, H. H., Kapranov, P., Sekinger, E. A., Kampa, D., and Gingeras, T. R. (2004). Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. Cell, 116(4), 499-509.

Chang J.C., Wooten E.C., Tsimelzon A., Hilsenbeck S.G., Gutierrez M.C., Elledge R., Mohsin S., Osborne C.K., Chamness G.C., Allred D.C., O'Connell P. (2003). Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. The Lancet, 362(9381), 362-369.

Cui X., Churchill G.A. (2003). Statistical tests for differential expression in cDNA microarray experiments.

Genome Biology, 4(4), 210.

Dai, M., Wang, P., Boyd, A. D., Kostov, G., Athey, B., Jones, E. G., and Luo, H. (2005). Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Research, 33(20), e175.

Ein-Dor L., Kela I., Getz G., Givol D., Domany E. (2006). Outcome signature genes in breast cancer: Is there a unique set? Bioinformatics, 22(2), 171-178.

Fischer, M. (2017). Census and evaluation of p53 target genes. Oncogene, 36(28), 3943-3956.

Flores, E. R., Sengupta, S., Miller, J. B., Newman, J. J., Bronson, R., Crowley, D., and Jacks, T. (2002). Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell, 1(1), 91-99.

Flores, E. R., Sengupta, S., Miller, J. B., Newman, J. J., Bronson, R., Crowley, D., and Jacks, T. (2002). Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell, 1(1), 91-99.

Flores, E. R., Sengupta, S., Miller, J. B., Newman, J. J., Bronson, R., Crowley, D., and Jacks, T. (2002). Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell, 2(4), 363-373.

Gebel, J., Luh, L. M., Coutandin, D., Osterburg, C., Löhr, F., Schäfer, B., and Dötsch, V. (2020). Mechanism of TAp73 inhibition by ΔNp63 and structural basis of p63/p73 hetero-tetramerization. Cell Death and Differentiation, 27(5), 1604-1619.

Geschwind, D. H., and Konopka, G. (2009). Neuroscience in the era of functional genomics and systems biology. Nature, 461(7266), 908-915.

Golub, T. R., Slonim, D. K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J. P., and Lander, E. S. (1999). Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science, 286(5439), 531-537.

Gorski, J. J., James, C. D., and Wahl, G. M. (2003). A high-throughput matrix-based screen for genomewide RNAi identifies susceptibility genes to herpes simplex virus. Genome Research, 13(10), 1974-1984.

He, Z., Gentry, T. J., Schadt, C. W., Wu, L., Liebich, J., Chong, S. C., and Zhou, J. (2010). GeoChip: a comprehensive microarray for investigating biogeochemical, ecological, and environmental processes. The ISME Journal, 1(1), 67-77.

Hibi K., Trink B., Patturajan M., Westra W.H., Caballero O.L., Hill D.E., Ratovitski E.A., Jen J., Sidransky

D. (2000). AIS is an oncogene amplified in squamous cell carcinoma. Proceedings of the National Academy of Sciences of the United States of America, 97(10), 5462-5467.

Hirschhorn, J. N., and Daly, M. J. (2005). Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics, 6(2), 95-108.

Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols, 4(1), 44-57.

Hughes, T. R., Marton, M. J., Jones, A. R., Roberts, C. J., Stoughton, R., Armour, C. D., and Friend, S. H. (2000). Functional discovery via a compendium of expression profiles. Cell, 102(1), 109-126.

Iorio, M. V., Ferracin, M., Liu, C. G., Veronese, A., Spizzo, R., Sabbioni, S., and Croce, C. M. (2016). MicroRNA gene expression deregulation in human breast cancer. Cancer Research, 65(16), 7065- 7070.

Irizarry, R. A., Warren, D., Spencer, F., Kim, I. F., Biswal, S., Frank, B. C., and Rifkin, S. A. (2003). Multiple- laboratory comparisons of microarray platforms. Nature Methods, 2(5), 345-350.

Joerger, A. C., and Fersht, A. R. (2016). The p53 Pathway: Origins, Inactivation in Cancer, and Emerging Therapeutic Approaches. Annual Review of Biochemistry, 85(1), 375–404.

Johnson, D. S., Mortazavi, A., Myers, R. M., and Wold, B. (2007). Genome-wide mapping of in vivo protein- DNA interactions. Science, 316(5830), 1497-1502.

Kadota K., Nakai Y., Shimizu K. (2003). A weighted average difference method for detecting differentially expressed genes from microarray data. Algorithms for Molecular Biology, 19(1), 8.

Kandoth C., McLellan M.D., Vandin F., Ye K., Niu B., Lu C., Xie M., Zhang Q., McMichael J.F., Wyczalkowski M.A., Leiserson M.D., Miller C.A., Welch J.S., Walter M.J., Wendl M.C., Ley T.J., Wilson R.K., Raphael B.J., Ding L. (2013). Mutational landscape and significance across 12 major cancer types. Nature, 502(7471), 333-339.

Kerr M.K., Churchill G.A. (2001). Statistical design and the analysis of gene expression microarray data.

Genetical Research, 77(2), 123-128.

Kosmidou, V., Katseli, V., Balatsos, N. A., Stathopoulos, C., Tzavaras, T., and Kyriakidis, D. A. (2019). p53 enhances ribosomal RNA transcription and recruits RNA Polymerase I to target promoters. Oncotarget, 10(45), 4660-4670

Koster, M. I., Kim, S., Mills, A. A., DeMayo, F. J., and Roop, D. R. (2007). p63 is the molecular switch for initiation of an epithelial stratification program. Genes and Development, 18(2), 126-131.

Kruse, J. P., and Gu, W. (2009). Modes of p53 regulation. Cell, 137(4), 609-622.

Lane, D. P., and Levine, A. J. (2010). p53 Research: the past thirty years and the next thirty years. Cold Spring Harbor Perspectives in Biology, 2(12), a000893.

Lee H.K., Hsu A.K., Sajdak J., Qin J., Pavlidis P. (2004). Coexpression analysis of human genes across many microarray data sets. Genome Research, 14(6), 1085-1094.

Leek J.T., Scharpf R.B., Bravo H.C., Simcha D., Langmead B., Johnson W.E., Geman D., Baggerly K., Irizarry R.A. (2010). Tackling the widespread and critical impact of batch effects in high-throughput data. Nature Reviews Genetics, 11(10), 733-739.

Levin J.Z., Yassour M., Adiconis X., Nusbaum C., Thompson D.A., Friedman N., Gnirke A., Regev A. (2016). Comprehensive comparative analysis of strand-specific RNA sequencing methods. Nature Methods, 7(9), 709-715.

Lockhart, D. J., Dong, H., Byrne, M. C., Follette, M. T., Gallo, M. V., Chee, M. S., and Davis, R. W. (1996). Expression monitoring by hybridization to high-density oligonucleotide arrays. Nature Biotechnology, 14(13), 1675-1680.

Luo J., Schumacher M., Scherer A., Sanoudou D., Megherbi D., Davison T., Shi T., Tong W., Shi L., Hong H., Zhao C., Elloumi F., Shi W., Thomas R., Lin S., Tillinghast G., Liu G., Zhou Y., Herman D., Li Y., Deng Y., Fang H., Bushel P., Woods M., Zhang J. (2009). A comparison of batch effect removal methods for enhancement of prediction performance using MAQC-II microarray gene expression data. The Pharmacogenomics Journal, 10(4), 278-291.

Melino, G., Memmi, E. M., Pelicci, P. G., and Bernassola, F. (2015). Maintaining epithelial stemness with p63. Science Signaling, 8(398), re9.

Menendez, D., Inga, A., and Resnick, M. A. (2011). The expanding universe of p53 targets. Nature Reviews Cancer, 9(10), 724-737.

Muller P.A., Caswell P.T., Doyle B., Iwanicki M.P., Tan E.H., Karim S., Lukashchuk N., Gillespie D.A., Ludwig R.L., Gosselin P., Cromer A., Brugge J.S., Sansom O.J., Norman J.C., Vousden K.H. (2009). Mutant p53 drives invasion by promoting integrin recycling. Cell, 139(7), 1327-1341.

Olivier, M., Hollstein, M., and Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harbor Perspectives in Biology, 2(1), a001008.

Pan Q., Shai O., Lee L.J., Frey B.J., Blencowe B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nature Genetics, 40(12), 1413-1415.

Perou C.M., Sørlie T., Eisen M.B., van de Rijn M., Jeffrey S.S., Rees C.A., Pollack J.R., Ross D.T., Johnsen H., Akslen L.A., Fluge Ø., Pergamenschikov A., Williams C., Zhu S.X., Lønning P.E., Børresen-Dale A.L., Brown P.O., Botstein D. (2000). Molecular portraits of human breast tumors. Nature, 406(6797), 747-752.

Perou, C. M., Sørlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., and Brown, P. O. (2000).

Molecular portraits of human breast tumors. Nature, 406(6797), 747-752.

Pinkel, D., Segraves, R., Sudar, D., Clark, S., Poole, I., Kowbel, D., and Albertson, D. (1998). High-resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nature Genetics, 20(2), 207-211.

Quackenbush, J. (2002). Microarray data normalization and transformation. Nature Genetics, 32(Supp1), 496- 501.

Ren, B., Robert, F., Wyrick, J. J., Aparicio, O., Jennings, E. G., Simon, I., and Myers, R. M. (2000). Genome-wide location and function of DNA binding proteins. Science, 290(5500), 2306-2309.

Riley T., Sontag E., Chen P., Levine A. (2008). Transcriptional control of human p53-regulated genes. Nature Reviews Molecular Cell Biology, 9(5), 402-412.

Rocco J.W., Leong C.O., Kuperwasser N., DeYoung M.P., Ellisen L.W. (2006). p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell, 9(1), 45-56.

Sakamuro, D., Sabbatini, P., White, E., and Prendergast, G. C. (1995). The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene, 11(12), 2379-2386.

Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science, 270(5235), 467-470.

Senoo, M., Pinto, F., Crum, C. P., and McKeon, F. (2007). p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell, 129(3), 523-536.

Shi, L., Campbell, G., Jones, W. D., Campagne, F., Wen, Z., Walker, S. J., and Tong, W. (2020). The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. Nature Biotechnology, 28(8), 827-838.

Slonim, D. K. (2002). From patterns to pathways: gene expression data analysis comes of age. Nature Genetics, 32(Supp1), 502-508.

Smith, A. M., Heller, M. J., Knoll, W., and Dorfman, K. D. (2008). Microarrays: Biophysical tools for unraveling the functional genome. Current Opinion in Chemical Biology, 12(5), 476-484.

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Statistical Applications in Genetics and Molecular Biology, 3(1), 1-25.

Su, X., and Flores, E. R. (2013). p63 steps into the limelight: crucial roles in the suppression of tumorigenesis and metastasis. Nature Reviews Cancer, 13(2), 136-143.

Su, X., Chakravarti, D., Cho, M. S., Liu, L., Gi, Y. J., Lin, Y. L., and Ellisen, L. W. (2009). TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. Nature, 467(7318), 986-990.

Tabaries, S., McNulty, A., Ouellet, V., Annis, M. G., Dessureault, M., Vinette, M., and Royal, I. (2021). Afadin cooperates with Claudin-2 to promote breast cancer metastasis. Genes, Chromosomes and Cancer, 60(8), 558-571.

van Gelder, R. N., von Zastrow, M. E., Yool, A., Dement, W. C., Barchas, J. D., and Eberwine, J. H. (1990). Amplified RNA synthesized from limited quantities of heterogeneous cDNA. Proceedings of the National Academy of Sciences, 87(5), 1663-1667.

van 't Veer L.J., Dai H., van de Vijver M.J., He Y.D., Hart A.A., Mao M., Peterse H.L., van der Kooy K., Marton M.J., Witteveen A.T., Schreiber G.J., Kerkhoven R.M., Roberts C., Linsley P.S., Bernards R., Friend S.H. (2002). Gene expression profiling predicts the clinical outcome of breast cancer. Nature, 415(6871), 530-536.

Vousden, K. H., and Prives, C. (2009). Blinded by the Light: The Growing Complexity of p53. Cell, 137(3), 413-431.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics, 10(1), 57-63.

Wilhelm, B. T., Marguerat, S., Watt, S., Schubert, F., Wood, V., Goodhead, I., and Bähler, J. (2010). Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. Nature, 464(7289), 250-255.

Xu, H., Wang, X., Liu, D. X., and Moro, A. (2014). Understanding the molecular mechanisms of the liver's evolution, structure, and function through genomics: Willkommen, bienvenue and welcome to RNAseq! Integrative and Comparative Biology, 54(2), 328-341.

Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dotsch, V., and McKeon, F. (1998). p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Molecular Cell, 2(3), 305-316.

Yang, A., Schweitzer, R., Sun, D., Kaghad, M., Walker, N., Bronson, R. T., and McKeon, F. (1999). p63 is essential for regenerative proliferation in limb, craniofacial, and epithelial development. Nature, 398(6729), 714-718.

Yoshihara K., Shahmoradgoli M., Martínez E., Vegesna R., Kim H., Torres-Garcia W., Treviño V., Shen H., Laird P.W., Levine D.A., Carter S.L., Getz G., Stemke-Hale K., Mills G.B., Verhaak R.G. (2013). Inferring tumor purity and stromal and immune cell admixture from expression data. Nature Communications, 4, 2612.

Zhang, B., Horvath, S., and Zhang, S. (2013). A general framework for weighted gene co-expression network analysis. Statistical applications in genetics and molecular biology, 4(1).