In Vitro Exploration of TP53 in Breast Cancer: Unlocking the Cellular Signaling Network

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Abstract:

In breast cancer, the tumor suppressor gene TP53 plays a pivotal role in regulating cell cycle arrest, apoptosis, and DNA repair, with dysregulation frequently observed in aggressive subtypes such as triple-negative breast cancer (TNBC) and HER2-positive tumors. In vitro models provide a controlled environment to dissect the complex cross-talk between TP53 and estrogen receptor (ER) signaling pathways, critical for understanding subtype-specific tumor biology and therapy resistance. This review consolidates evidence from cellular studies utilizing diverse breast cancer cell lines, including MCF-7 and MDA-MB-231, employing assays such as MTT viability, wound-healing migration, and Western blot analysis to assess TP53 inhibition effects and downstream signaling alterations. Furthermore, network pharmacology approaches that predict phytochemical modulators targeting the TP53-ER axis are explored, highlighting compounds like curcumin and resveratrol with potential therapeutic synergy. Integrating in vitro assay results with computational predictions advances our understanding of TP53-driven signaling networks and aids in identifying novel agents for tailored breast cancer treatment. The review concludes by emphasizing the translational prospects of these findings, advocating for the development of advanced in vitro models and multi-omics integration to refine targeted therapies in breast cancer subtypes, ultimately improving clinical outcomes.

Keywords: TP53, breast cancer, estrogen receptor, in vitro models, network pharmacology, triple-negative breast cancer, phytochemicals, HER2-positive breast cancer.

Introduction:

Breast cancer is a heterogeneous disease comprising multiple subtypes defined by distinct molecular and clinical characteristics, which significantly influence therapeutic responses and prognoses.[1] The three major subtypes—triple-negative breast cancer (TNBC), HER2-positive breast cancer, and estrogen receptor (ER)-positive breast cancer—exhibit distinct profiles of gene expression, receptor status, and mutational landscapes. Among the critical molecular players implicated in breast cancer pathogenesis, the tumor suppressor gene TP53 stands out for its frequent mutation and functional dysregulation.[2] TP53 mutations are prevalent in approximately 80% of TNBC cases, highlighting its central role in driving the aggressive phenotype of this subtype, while aberrations in TP53 also contribute to progression in HER2-positive and, to a lesser extent, ER-positive breast cancers. Understanding TP53 dysregulation in these subtypes is essential, given its profound impact on cell fate decisions and therapeutic resistance.[3]

TP53 encodes the p53 protein, often termed the “guardian of the genome,” which orchestrates cellular responses to stress through mechanisms including cell cycle arrest, apoptosis, and DNA damage repair.[4] The wild-type p53 protein functions as a transcription factor, regulating genes involved in maintaining genomic stability and suppressing tumorigenesis. In contrast, mutant forms of TP53 frequently lose tumor suppressor capabilities and may gain oncogenic functions that promote cancer progression, metastasis, and resistance to standard therapies. This duality underscores the complexity of TP53’s role in breast cancer biology and necessitates detailed mechanistic investigation.[5]

Estrogen receptor signaling further complicates the molecular landscape of breast cancer. ER-positive breast cancers rely on estrogen binding to ER-α and ER-β isoforms to drive proliferation and survival.[6] Notably, TP53 and ER pathways exhibit intricate cross-talk mechanisms influencing each other's expression and activity. For instance, wild-type p53 can modulate ER target gene expression, while ER signaling can impact TP53 transcriptional activity. This interplay affects cellular phenotypes, chemoresistance, and disease outcomes, particularly in ER-positive and

HER2-positive contexts. Dissecting this cross-talk is critical to developing new therapeutic strategies that effectively target these interacting pathways.[7]

In vitro models provide a strategic advantage for such investigations. Unlike in vivo systems, cell-based assays and culture systems offer controlled experimental environments that enable manipulation of specific genes and pathways with precision.[8] Commonly used breast cancer cell lines such as MCF-7 (ER-positive), MDA-MB-231, and MDA-MB-468 (TNBC subtypes with TP53 mutations) afford valuable platforms to study TP53 and ER dynamics. Additionally, advances in 3D cultures and organoid models allow for more physiologically relevant investigations of tumor biology, while CRISPR/Cas9 gene-editing technologies facilitate precise modulation of TP53 and ER signaling components. These in vitro approaches are indispensable for elucidating molecular cross-talk, validating pharmacological interventions, and screening phytochemicals computationally predicted to modulate TP53-ER networks via network pharmacology.[9]

This review aims to synthesize current in vitro evidence elucidating the TP53-estrogen receptor cross-talk in breast cancer, focusing on the TNBC and HER2-positive subtypes noted for TP53 alterations. It will critically evaluate cellular models and experimental assays employed to assess TP53 inhibition and functional outcomes, including proliferation, migration, and protein expression. Furthermore, the review will cover network pharmacology methodologies used to identify phytochemical candidates that target TP53-related signaling pathways, bridging computational predictions with experimental validations.[10]

TP53 Overexpression in Triple-Negative and HER2-Positive Breast Cancer Subtypes

Breast cancer subtype-specific alterations in TP53 expression and function significantly impact tumor aggressiveness and treatment response. This section delves into the overexpression and mutational landscape of TP53 in triple-negative breast cancer (TNBC) and HER2-positive breast cancer subtypes, highlighting the molecular mechanisms driving tumor progression, supported by evidence from in vitro studies. It then contrasts these features with ER-positive breast cancer, underscoring the implications for therapy resistance.[11]

TP53 in TNBC:

Triple-negative breast cancer is characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). This highly aggressive subtype accounts for approximately 15–20% of breast cancer cases and is notorious for poor prognosis due to limited targeted therapies and frequent resistance to conventional chemotherapy.[12] Studies report TP53 mutations or overexpression in nearly 80% of TNBC cases, making TP53 aberrations a hallmark of this subtype. The predominance of mutant TP53 confers loss of tumor suppressor functions, enabling unchecked proliferation, evasion of apoptosis, and enhanced metastatic potential.[13]

Mechanistically, TP53 mutations in TNBC lead to gain-of-function oncogenic activities. Mutant p53 proteins can interfere with transcriptional regulation, promote genomic instability, and modulate cellular pathways contributing to epithelial-to-mesenchymal transition (EMT), chemoresistance, and stemness.[14] In vitro investigations using TNBC cell lines such as MDA-MB-231 and MDA-MB-468 have elucidated the role of mutant TP53 in driving aggressive phenotypes. For example, silencing mutant TP53 in MDA-MB-231 reduces invasiveness and proliferation, while overexpression promotes migratory behavior and resistance to apoptosis-inducing agents. These findings underscore TP53 as a potential therapeutic target in TNBC.[15]

TP53 in HER2-Positive Breast Cancer:

HER2-positive breast cancers, constituting about 15–25% of cases, display amplification and overexpression of the HER2 receptor tyrosine kinase, which activates downstream proliferative and survival signaling pathways. TP53 alterations are present in a substantial subset of HER2-positive tumors, often co-occurring with HER2 amplification. The synergistic interaction between TP53 dysfunction and HER2-driven signaling contributes to enhanced oncogenicity and therapy resistance.[16]

Studies indicate that mutant TP53 can enhance HER2-mediated activation of pathways such as phosphatidylinositol 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK), fostering cell survival and proliferation.[17] In vitro, HER2-amplified cell lines harboring TP53 mutations exhibit heightened resistance to HER2-targeted therapies like trastuzumab, linked to activation of compensatory signaling and reduced apoptosis. For instance, in HER2-positive cell models, TP53 mutation correlates with increased levels of phosphorylated AKT, which promotes survival signals, rendering cells less responsive to therapeutic agents. These results highlight TP53 status as a critical modulator of HER2-positive breast cancer biology and treatment outcomes.[18]

Comparative Analysis with ER-Positive Subtypes:

In contrast to TNBC and HER2-positive breast cancers, ER-positive subtypes generally harbor fewer TP53 mutations, with mutation frequencies around 20–30%. The presence of wild-type TP53 in many ER-positive tumors facilitates cell cycle regulation and apoptosis, contributing to better prognosis and responsiveness to hormone therapies.[19] Importantly, the interplay between ER signaling and TP53 in ER-positive cancers is complex, with evidence suggesting that functional TP53 can suppress ER-driven transcriptional programs, while ER may reciprocally regulate TP53 activity, balancing proliferation and cell death.[20]

The relative scarcity of TP53 mutations in ER-positive subtypes partially explains their distinct therapeutic profiles and resistance mechanisms compared to TNBC and HER2-positive cancers. However, when TP53 mutations do occur in ER-positive tumors, they are often associated with endocrine therapy resistance and more aggressive behavior. This contrast underscores the pivotal role of TP53 status in subtype-specific tumor biology.[21]

Implications for Therapy Resistance:

TP53 overexpression and mutation contribute robustly to therapy resistance across breast cancer subtypes but with distinct mechanisms. In TNBC, mutant TP53 supports resistance to DNA-damaging agents by impairing apoptosis and promoting repair evasion. Similarly, in HER2-positive cancers, TP53 dysfunction synergizes with HER2 signaling to activate survival pathways counteracting targeted therapies. Conversely, ER-positive cancers with intact TP53 often respond well to hormone-based treatments, whereas mutations may herald resistance.[22]

In Vitro Models Revealing TP53-ER Cross-Talk:

In vitro models are instrumental for dissecting the complex molecular interplay between TP53 and estrogen receptor (ER) signaling pathways in breast cancer. These systems enable detailed investigation of cellular mechanisms underpinning TP53-ER cross-talk, providing critical insights into tumor biology and informing therapeutic strategies.[23] This section discusses commonly used cellular models, elucidates the mechanistic basis of TP53-ER interactions, reviews key in vitro evidence supporting their modulation, and addresses challenges with current models alongside emerging technological advancements.[24]

Cellular Models:

The choice of cellular models greatly influences the translational relevance of in vitro studies. Among breast cancer cell lines, MCF-7, an ER-positive and wild-type TP53-expressing line, serves as a quintessential model to study ER-driven pathways and their regulatory interactions with functional TP53. Conversely, MDA-MB-468—representative of triple-negative breast cancer (TNBC)—harbors mutant TP53 and lacks ER expression, enabling exploration of TP53 dysfunction in a hormone receptor-negative context.[25]

Beyond traditional 2D monolayer cultures, which allow high-throughput assays and ease of genetic manipulation, 3D culture systems including spheroids and organoids have gained prominence for better recapitulating in vivo tumor architecture, cell-cell interactions, and microenvironmental factors influencing TP53 and ER signaling. Organoid models, derived from patient tumors, further enhance clinical relevance by preserving intratumoral heterogeneity and receptor status, delivering more predictive insights into TP53-ER cross-talk dynamics.[26]

Mechanisms of Cross-Talk:

At the molecular level, TP53 and ER pathways engage in multifaceted cross-talk involving direct and indirect interactions that regulate transcriptional and post-translational events, ultimately modulating proliferation, apoptosis, and therapy response. Direct interactions include TP53 binding to estrogen response elements (EREs) within the genome, influencing ER target gene expression. TP53's transcriptional activity can suppress ER-mediated proliferation by modulating genes involved in cell cycle arrest and apoptosis.[27]

Indirect regulatory mechanisms involve intermediary proteins such as MDM2, an E3 ubiquitin ligase that governs TP53 stability and is itself transcriptionally regulated by ER. This creates feedback loops where ER signaling can modulate TP53 activity by altering MDM2 expression, and TP53 can reciprocally influence ER signaling outputs. Another indirect mediator is p63, a TP53 family member that interacts with both TP53 and ER pathways, contributing to cellular differentiation and survival signals.[28]

Downstream signaling cascades further illustrate this interplay. For example, TP53 influences MAPK and NF-κB pathways, which intersect with ER signaling to coordinately regulate cellular outcomes such as proliferation and inflammatory responses. These complex networks underscore the dynamic regulatory environment governed by TP53-ER cross-talk.[29]

In Vitro Evidence:

Multiple key studies using the aforementioned cellular and culture models illustrate these mechanisms. Reactivation of wild-type TP53 in ER-positive MCF-7 cells, through genetic or pharmacological means, has been shown to restore sensitivity to ER-targeted therapies by repressing pro-proliferative ER target genes and inducing apoptotic pathways.[30] For instance, treatment with TP53-reactivating agents led to decreased ER-driven proliferation and increased expression of TP53 downstream effectors like p21, demonstrating functional restoration of TP53-ER regulatory balance.[31]

In TNBC models such as MDA-MB-468, mutation-induced TP53 loss-of-function correlates with absent ER expression and enhanced aggressive behavior; however, emerging data show that modulating TP53 status can influence expression of ER-related signaling intermediates, suggesting latent regulatory axes. Studies applying CRISPR technology to edit mutant TP53 restored partial tumor suppressor functions, resulting in altered signaling through MAPK and NF-κB pathways that indirectly interact with ER signaling networks.[32]

Additionally, combined treatments targeting TP53 pathways and ER signaling demonstrated synergistic effects in cell viability reduction, migration inhibition, and apoptosis induction—highlighting translational potential. Advanced 3D culture systems corroborate these findings with more physiologically relevant responses, supporting the clinical applicability of targeting the TP53-ER axis.[33]

Challenges in Modeling:

Despite the progress in elucidating TP53-ER cross-talk via in vitro models, several challenges remain. Immortalized cell lines pose limitations due to genetic drift, artificial selection pressures, and lack of tumor microenvironmental complexity. These factors can bias signaling pathway activities and responsiveness to modulation, potentially skewing interpretations.[34]

Furthermore, conventional 2D cultures inadequately replicate 3D architectural cues influencing receptor signaling, cell polarity, and extracellular matrix interactions. While 3D spheroids and organoids offer improvements, they are more technically demanding, less standardized, and have throughput constraints.[35]

Emerging CRISPR/Cas9 genome editing techniques provide precise tools to introduce or correct TP53 mutations within relevant cell models, facilitating controlled studies of mutation-specific effects on ER cross-talk. Moreover, co-culture systems integrating stromal and immune components are being developed to more comprehensively simulate the tumor microenvironment's role in modulating TP53 and ER interactions.[36]

Cell-Based Assays for Assessing TP53 Inhibition in Breast Cancer:

Overview of Assays:

Cell-based assays provide critical tools for investigating TP53 inhibition and its functional consequences in breast cancer cell models. The primary endpoints include assessments of cell viability, migration/invasion capacity, and expression/phosphorylation status of key proteins involved in signaling pathways.[37] These endpoints allow researchers to elucidate the effects of TP53 perturbation on cellular proliferation, motility, and molecular signaling, thereby unraveling tumor suppressor roles and therapeutic susceptibility. The choice of assays reflects the multifaceted roles of TP53—from growth regulation to modulation of epithelial-to-mesenchymal transition (EMT)—and their dynamic interplay with estrogen receptor (ER) pathways.[38]

MTT Assay:

Among viability assays, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is widely used due to its simplicity, reproducibility, and quantitative readout based on mitochondrial metabolic activity.[39] Protocols typically involve seeding breast cancer cells (e.g., MCF-7, MDA-MB-231), treatment with TP53 modulators or gene silencing constructs, followed by incubation with MTT reagent. Metabolically active cells convert MTT to insoluble formazan crystals, which are solubilized and quantified spectrophotometrically.[40]

Applications of the MTT assay in TP53 knockdown or overexpression studies have elucidated TP53's role in regulating breast cancer cell proliferation. For instance, treatment with the MDM2 antagonist Nutlin-3 leads to reactivation of wild-type TP53, resulting in decreased viability in TP53-wild-type MCF-7 cells but limited effects in TP53-mutant TNBC lines.[41] Similarly, siRNA-mediated TP53 knockdown promotes viability in otherwise TP53-competent cells, demonstrating TP53's tumor suppressive function. The dose-response data from MTT assays guide effective concentrations for TP53 inhibitors and aid in screening novel compounds targeting TP53 pathways.[42]

Wound-Healing Assay:

Cell migration and invasion, pivotal for metastasis, are efficiently assessed using the wound-healing (scratch) assay. In this method, a confluent monolayer of breast cancer cells is artificially "wounded" with a pipette tip, creating a cell-free gap. The rate of gap closure over time reflects migratory capacity. This assay is particularly relevant for studying TP53-ER cross-talk in processes like EMT, where TP53 loss often promotes mesenchymal transition and enhanced migration.[43]

In vitro evidence indicates that TP53 inhibition or mutation enhances migration and invasiveness, observable as accelerated wound closure rates. Conversely, TP53 reactivation or overexpression reduces migratory behavior and modulates expression of EMT markers such as E-cadherin and vimentin, effects often further influenced by ER signaling status. The wound-healing assay thus provides a functional readout correlating with molecular changes in TP53-ER pathways affecting metastatic potential.[44]

Western Blot and Related Techniques:

Western blotting remains a cornerstone technique for detecting TP53 protein levels, post-translational modifications (notably phosphorylation status indicative of activation), and ER expression. Cells subjected to TP53-modulating treatments or genetic interventions undergo protein extraction, separation by SDS-PAGE, transfer to membranes, and immunodetection using specific antibodies.[45]

Integration of western blot data with quantitative PCR (qPCR) and enzyme-linked immunosorbent assays (ELISA) facilitates comprehensive signaling pathway analyses. For example, western blot analysis can monitor phosphorylation of TP53 at Ser15 or Ser20—sites linked to DNA damage response—alongside ER-α expression changes.[46] Concurrent qPCR assays quantify downstream transcript levels of TP53 target genes (e.g., p21, BAX), while ELISA may measure secreted factors modulated by TP53 and ER pathways.[47]

These combined molecular assays provide mechanistic insights into functional consequences of TP53 inhibition, including effects on cell cycle regulation, apoptosis induction, and ER downstream signaling networks.[48]

Integration and Validation:

Robust assessment of TP53 inhibition necessitates combining multiple assays to capture cellular phenotypes and molecular mechanisms holistically. For example, viability data from MTT assays paired with migration results from wound-healing studies and protein expression profiles from western blots yield multidimensional understanding of TP53's role.[49]

Recent developments integrate high-throughput adaptations such as siRNA or CRISPR screens targeting TP53 and associated pathways, enabling systematic interrogation of gene function across diverse breast cancer models. Such screens paired with automated imaging and multiplexed readouts streamline discovery of novel TP53 modulators and define context-specific effects on ER cross-talk.[50]

Network Pharmacology for Predicting Phytochemicals Targeting TP53 Signaling

Principles of Network Pharmacology:

Network pharmacology represents a paradigm shift from traditional "one drug, one target" approaches to a holistic multi-target strategy, especially relevant in complex diseases like breast cancer involving intricate signaling networks such as those governed by TP53. This approach harnesses systems biology and bioinformatics tools to map the interactions among genes, proteins, metabolites, and drugs, identifying key nodes and pathways susceptible to modulation.[51] For TP53 signaling, database resources like STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) provide protein-protein interaction networks, while TCMSP (Traditional Chinese Medicine Systems Pharmacology Database) and others offer phytochemical and target data critical for screening natural compounds' effects on TP53 and related pathways.[52]

By integrating these databases, network pharmacology allows the construction of robust interactomes linking TP53 with estrogen receptor (ER) signaling and other oncogenic pathways, enabling identification of candidate phytochemicals that can bind or modulate multiple targets within these networks. This multi-dimensional analysis is vital to uncover compounds with potential to exert synergistic or additive effects on breast cancer cells, overcoming resistance mechanisms rooted in pathway redundancy.[53]

Phytochemical Identification:

Screening phytochemicals for engagement with the TP53-ER network begins with compiling natural compound libraries enriched with bioactive molecules such as curcumin, resveratrol, quercetin, and epigallocatechin gallate (EGCG). These agents have demonstrated pleiotropic anti-cancer properties, including modulation of TP53 function and ER signaling.[54]

In silico methods such as molecular docking simulate binding affinity between phytochemicals and target proteins within the TP53 network, forecasting potential interactions at active or allosteric sites. For instance, curcumin has shown binding capabilities to MDM2, an antagonist of TP53, suggesting restoration of p53 activity by inhibiting its degradation. Similarly, resveratrol and quercetin have been predicted to interact with TP53 and key nodes in ER pathways, indicating their influence on cross-talk signaling.[55]

In Vitro Validation:

Predicted phytochemical hits necessitate empirical validation using breast cancer cell lines that model TP53-ER cross-talk, including MCF-7 (ER-positive, wild-type TP53) and TNBC lines like MDA-MB-231 (mutant TP53, ER-negative). Cell viability assays (e.g., MTT), apoptosis markers, and protein expression analyses confirm the functional impact of phytochemicals on TP53 activation and downstream signaling.[56]

For example, docking predictions pinpointing curcumin's interaction with MDM2 have been substantiated by in vitro evidence showing enhanced TP53 stabilization and increased expression of pro-apoptotic genes upon curcumin treatment. Combination treatments with phytochemicals and chemotherapy drugs demonstrate amplified cytotoxic effects in TP53-mutant TNBC models by potentiating DNA damage responses and inhibiting survival pathways like PI3K/AKT.[57]

Moreover, molecular assays reveal modulation of ER expression and phosphorylation by certain phytochemicals, indicative of their capacity to influence TP53-ER cross-talk beyond TP53 alone. These validations bridge computational predictions with biological relevance, establishing the feasibility of phytochemical-based interventions targeting TP53 regulatory networks.[58]

Therapeutic Potential:

Network pharmacology-guided phytochemical identification opens avenues for novel therapeutics with enhanced efficacy and reduced toxicity profiles. The multi-target nature of phytochemicals addresses the complex resistance mechanisms in breast cancer subtypes, particularly in TNBC and HER2-positive tumors where TP53 dysfunction is prevalent.[59]

Synergistic effects between phytochemicals and conventional chemotherapeutics have been documented. For instance, resveratrol augments doxorubicin efficacy in TNBC cells by modulating TP53-mediated apoptosis, while curcumin enhances trastuzumab sensitivity in HER2-positive models by disrupting survival signaling. These combinatorial regimens capitalize on phytochemicals' ability to modulate multiple nodes in TP53-ER networks, thereby overcoming single-agent resistance.[60]

Conclusion:

The exploration of TP53 and ER cross-talk through in vitro models has significantly advanced our understanding of breast cancer biology, especially in subtypes like TNBC and HER2-positive tumors where TP53 dysregulation plays a central role. These models—ranging from traditional 2D cultures to sophisticated 3D spheroids and organoids—have provided invaluable insights into the molecular mechanisms governing tumor progression, therapy resistance, and the complex signaling networks involving TP53 and ER pathways. Cell-based assays, including viability, migration, and protein expression analyses, have been instrumental in validating the functional impact of TP53 modulation, enabling identification of potential therapeutic targets.

Furthermore, the integration of network pharmacology has opened new avenues for discovering natural phytochemicals capable of targeting TP53-related pathways. In silico predictions validated through rigorous in vitro experiments underscore the potential of compounds like curcumin and resveratrol to restore TP53 activity, enhance chemosensitivity, and overcome resistance mechanisms.

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