Review Article

A review on A549 cell line in Non-Small-Cell lung cancer: Molecular pathways, drug resistance and emerging therapeutic strategies

Kannan R.*, Natarajan P., Ganesh H., Dinesh Siva N., Indhuja R.

Sankaralingam Bhuvaneswari College of Pharmacy, Anaikuttam, Sivakasi, Tamil Nadu, India

Affiliated with Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India

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Abstract

To date, non-small-cell lung cancer (NSCLC) still accounts for most cancer-related mortality, making an efficient model a pressing need for a comprehensive approach. The human lung adenocarcinoma-derived A549 cell line has been widely used for research in NSCLC due to cell reproducibility, KRAS mutation, and involvement of key oncogenic signaling. The A549 cell line emphasizes important aspects of cancer biology, namely apoptosis resistance, metabolism, and sustained proliferation driven by PI3K/AKT/mTOR, NF-κB, and KRAS/RAF/MEK/ERK. Oncogenic KRAS expression and NF-κB-mediated transcription induce chemoresistance through increased expression of efflux transporters (MDR1) and anti-apoptotic proteins (BCL-2, XIAP). In addition, A549 cell monolayers maintain cancer stem-like populations (e.g., CD90+ and high-ALDH1 cells), sustaining cancer growth and relapse. Innovative strategies support A549 model applications. CRISPR-Cas9 functional genomics facilitates genetic vulnerability mapping, while 3D cell cultures and organoids model a cancer microenvironment. Integration with artificial intelligence (AI) technology also helps to facilitate predictive models and screening of medications. A549, despite limitations like the lack of patient heterogeneity and the simplification of the microenvironment, is extremely useful in combination with other models or systems. In conclusion, A549 remains at the forefront, integrating knowledge and innovation to promote precise oncology to ensure NSCLC treatment efficacy.

***Keywords*:** A549 cell line, non-small-cell lung cancer, KRAS, PI3K/AKT/mTOR, NF-κB, drug resistance, cancer stem cells, CRISPR, organoids, artificial intelligence

Introduction

With around 1.8 million deaths per year and nearly 18 percent of all cancer deaths, lung cancer continues to be the world's largest cause of cancer-related mortality. (Sung et al., 2021). Non-small-cell lung cancer (NSCLC), which includes adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma, accounts for around 85% of its histological subtypes (Herbst et al., 2018). NSCLC still has a low overall survival rate despite advancements in molecular diagnostics and targeted therapy. This is mostly because of late-stage diagnosis, significant metastatic potential, and the development of resistance to both conventional and targeted treatments (Howlader et al., 2020; Molina et al., 2008). To overcome these challenges, studies of the biology of illness and treatment results require robust preclinical models. Despite providing valuable insights in vivo, animal models are limited by interspecies variation, cost, and ethical considerations (Johnson et al., 2001). Therefore, in vitro cancer cell lines continue to be essential resources, providing repeatable, affordable, and thoroughly described systems for investigating tumour biology, signalling pathways, and treatment response (Mirabelli et al., 2019). One of the most commonly used NSCLC cell lines in cancer research is the A549 cell line, which was first created in 1972 by Giard and associates from the lung tissue of a male patient with adenocarcinoma who was 58 years old (Giard et al., 1973). Its altered TP53 function, KRAS mutations, and epithelial morphology make it extremely useful for simulating the course of lung cancer, drug resistance, and treatments (Gazdar et al., 2010; Vaughan et al., 2006). A549 cells are important for reasons other than only drug screening. They

Address for Corresponding Author:
Kannan R.
Department of Pharmacology
Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamil Nadu, India
Email: Kannanravi3873@gmail.com

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have played a crucial role in analysing important molecular pathways that control tumour cell proliferation, survival, and resistance to treatment, such as PI3K/AKT, NF-κB, and ROS-mediated apoptosis. Additionally, A549 cells are used as models to investigate drug resistance mechanisms, such as the role of cancer stem cell (CSC) populations in therapeutic relapse and efflux pump activity and epithelial–mesenchymal transition (EMT) (Ikeda et al., 2011; Yan et al., 2013). More recently, developments like 3D organoid culture techniques, CRISPR-Cas9 gene editing, and artificial intelligence (AI)-based predictive models have increased the translational potential of A549 research, allowing for more precisely targeted and physiologically relevant applications (Cunniff et al., 2021; Doench, 2018; H. Kim et al., 2020; Vamathevan et al., 2019).

In this study, we provide a overview of the function of A549 cells in NSCLC research, focusing on three interconnected domains: (i) Molecular pathways that control tumour survival and response to therapy; (ii) drug resistance and CSC dynamics that compromise treatment effectiveness; and (iii) new therapeutic approaches that combine A549-based models with next-generation technologies. By combining these viewpoints, we hope to draw attention to the advantages and disadvantages of A549 cells as a translational platform and to suggest potential future uses for them in customised oncology.

Characteristics of A549 cell line

Morphology and Chromosomal Profile

The A549 cell line, which grows as adherent monolayers in culture with polygonal, squamous-shaped cells, is well known for having an epithelial-like appearance (Shapiro et al., 1978). They have lamellar bodies, which are organelles that store pulmonary surfactant phospholipids, and other ultrastructural traits in common with human type II alveolar epithelial cells. Because these lamellar bodies can release phospholipids linked to surfactants into the extracellular environment, A549 is very pertinent for research on pulmonary biology and cancer. A549 cells are hypotriploid, according to karyotypic investigations, with a modal chromosome number of 66; nevertheless, there is significant variation among various passages and subclones (Mitelman et al., 2007). Their translational value is strengthened by the fact that this genomic instability reflects the significant chromosomal heterogeneity frequently seen in clinical NSCLC samples. Additional evidence of frequent chromosomal gains at 3q, 5p, and 8q and losses at 3p, 9p, and 17p—regions that include numerous lung cancer driver genes—is provided by comparative genomic hybridization and SNP array analysis (Collisson et al., 2014). Because of these changes, A549 serves as a representative platform for examining how chromosomal instability contributes to tumour growth.

Biochemical and Metabolic Features

A549 cells maintain a number of the specific biochemical roles of alveolar epithelial cells. One characteristic metabolic characteristic of type II pneumocytes is their ability to synthesise lecithin (phosphatidylcholine) through the cytidine diphosphocholine (Kennedy) pathway (Shapiro et al., 1978). They are a valuable system for examining the metabolism of lung surfactants and the impact of pharmaceutical or environmental factors on pulmonary function because of this characteristic. The metabolic phenotype of A549 cells is Warburg-like, with a strong dependence on aerobic glycolysis even when oxygen is present. High levels of lactate release and glucose uptake have been observed, which correspond to metabolic changes in NSCLC tumours (Shapiro et al., 1978; Srivastava et al., 2011). They also demonstrate enhanced activity of cytochrome P450 enzymes such as CYP1A1, CYP2B6, and CYP3A4, which are key in xenobiotic metabolism (Shapiro et al., 1978). A549 is hence appropriate for pharmacokinetic modelling, carcinogen activation, and drug toxicity investigations. Their tendency to produce reactive oxygen species (ROS) under stressful circumstances, like exposure to nanoparticles or medication treatment, is another noteworthy biochemical characteristic. High quantities of ROS can cause apoptosis and damage to the mitochondria, whereas low to moderate levels can encourage cell survival and growth (Srivastava et al., 2011). This duality makes A549 a versatile tool for redox biology and therapeutic intervention research.

Relevance to NSCLC Biology

A549 cells have a number of driver mutations on the genomic level that are commonly seen in human non-small cell lung cancer. The most notable is the activating KRAS mutation at codon 12 (G12S), which causes the RAS/MAPK signalling cascade to be constitutively activated, hence boosting unchecked cell growth, survival, and resistance to targeted treatments. This mirrors the clinical observation that KRAS mutations are present in ~25–30% of lung adenocarcinomas, particularly in smokers (Collisson et al., 2014). In addition, A549 cells exhibit mutational inactivation and dysregulation of TP53, the most commonly mutated tumor suppressor gene in NSCLC (Takahashi et al., 1989). TP53 dysfunction contributes to impaired DNA repair, evasion of apoptosis, and genomic instability, thereby complementing KRAS-driven oncogenesis. Transcriptomic profiling of A549 has also shown deregulation in other cancer-relevant genes, including EGFR, KEAP1, and STK11, further strengthening their clinical relevance (Collisson et al., 2014). Taken together, the combination of morphological similarity to alveolar epithelium, biochemical features of

surfactant metabolism and oxidative stress, and oncogenic mutations in KRAS and TP53 positions A549 as an extremely typical in vitro model of human NSCLC. These properties not only support their widespread use in cancer biology but also underscore their value in drug screening, toxicity testing, and the exploration of resistance mechanisms.

Key molecular pathways in A549 cells

The use of A549 cells as a translational model in non-small-cell lung cancer (NSCLC) research requires an understanding of the molecular signalling networks that control their behaviour. These pathways support therapeutic resistance mechanisms in addition to coordinating cellular survival, proliferation, and metastasis. Reactive oxygen species (ROS)-mediated processes, the NF-κB signalling cascade, and the PI3K/AKT/mTOR axis are among the many routes that have been studied. When taken as a whole, they shed light on oncogenic transformation and the results of NSCLC treatment.

PI3K/AKT/mTOR Pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway is a central regulator of growth and survival in A549 cells. Upon activation by receptor tyrosine kinases (RTKs) such as EGFR, PI3K generates phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which recruits AKT to the plasma membrane for activation(Vara et al., 2004). Activated AKT phosphorylates downstream effectors, including mTOR, glycogen synthase kinase-3β (GSK-3β), and BAD, collectively promoting proliferation, inhibition of apoptosis, and metabolic reprogramming (Engelman, 2009). A549 cells, harboring KRAS mutations, exhibit constitutive activation of PI3K/AKT signaling, which has been linked to resistance against EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib (Yao et al., 2010). Pharmacological inhibition of PI3K or mTOR has demonstrated significant growth suppression in A549 cultures, underscoring the therapeutic relevance of this pathway (Qiang et al., 2025). Moreover, studies integrating dual blockade of PI3K/AKT and MEK/ERK cascades indicate synergistic cytotoxicity, reflecting the crosstalk between these two major oncogenic drivers in NSCLC (Faber et al., 2009). A schematic representation of the PI3K/Akt pathway is shown in Figure 1.

NF-κB Signaling

In lung cancer, the nuclear factor-kappa B (NF-κB) pathway is a master regulator of immune evasion, cell survival, and inflammation. In A549 cells, inflammatory microenvironmental stimuli and oncogenic KRAS signalling frequently constitutively activate NF-κB (Karin, 2009). Under resting conditions, NF-κB dimers are sequestered in the cytoplasm by IκB inhibitors. Upon activation through cytokines (e.g., TNF-α, IL-1β), IκB kinase (IKK) phosphorylates IκB, leading to its degradation and subsequent nuclear translocation of NF-κB (Hayden and Ghosh, 2011). In A549 cells, NF-κB activation enhances the transcription of anti-apoptotic genes such as BCL-2 and XIAP, and upregulates drug efflux transporters

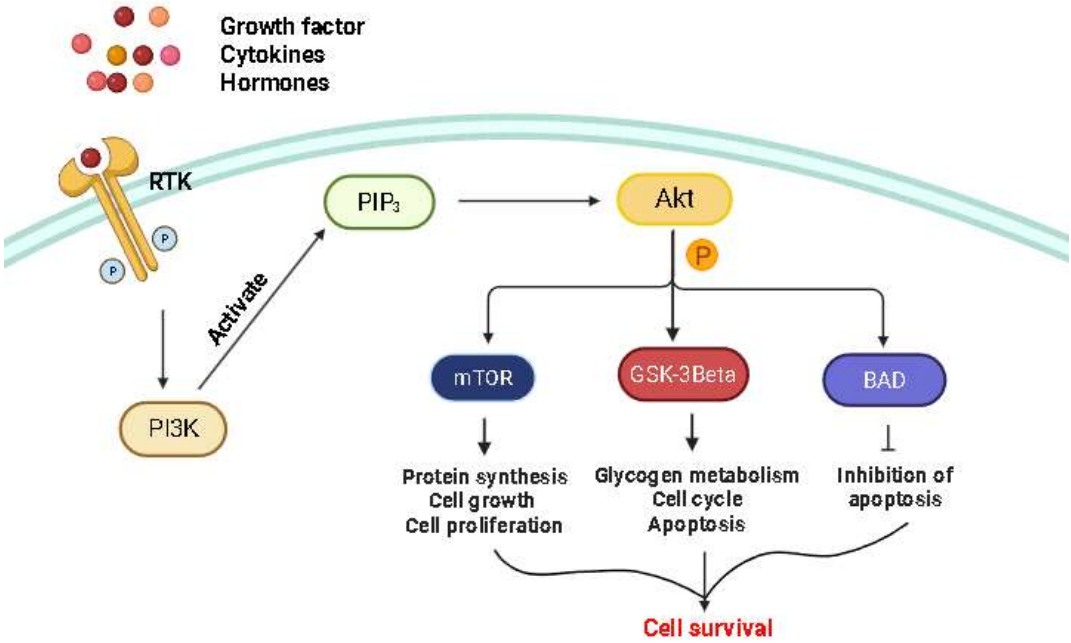

Figure 1: The P13K/Akt pathway integrates growth, metabolism, and apoptosis inhibition to ensure cell survival (Created in: https://BioRender.com)

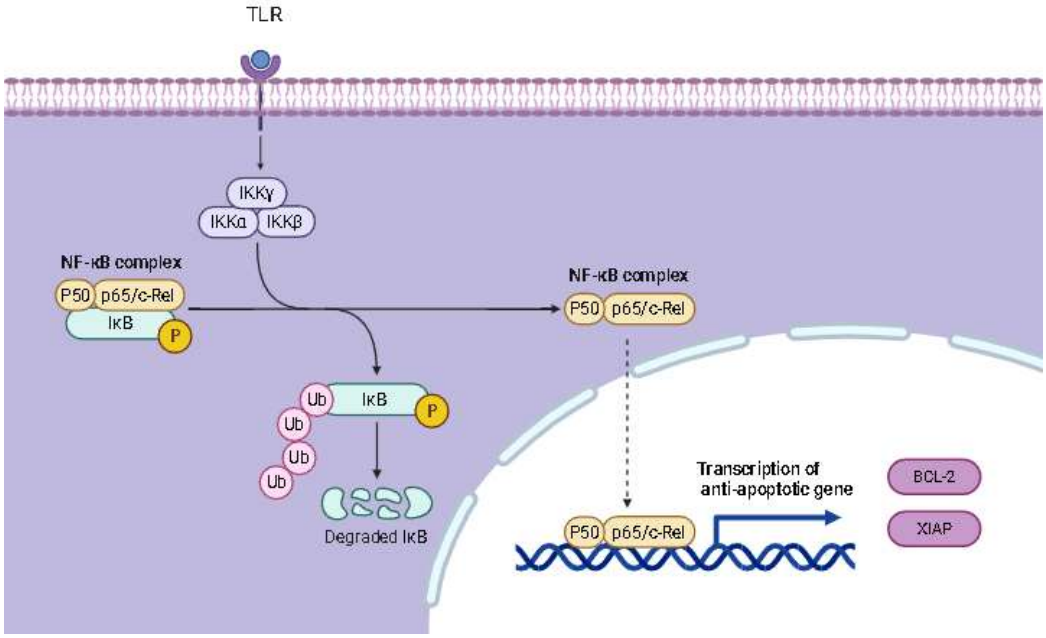

Figure 2: Mechanistic pathway of TLR- Driven NF-kB activation and anti-apoptotic transcription (Created in: https://BioRender.com).

like MDR1/P-glycoprotein, thereby contributing to chemoresistance (Bentires-Alj et al., 2003). Inhibition of NF-κB has been shown to sensitize A549 cells to cisplatin and radiation, highlighting its role as a mediator of therapy resistance. Furthermore, NF-κB interacts with other pathways, particularly PI3K/AKT and STAT3, amplifying pro-survival signals in NSCLC (Hoesel and Schmid, 2013). The TLR-driven NF-κB pathway contributes to enhanced cell survival under inflammatory conditions (Figure 2).

ROS-Mediated Mechanisms

Reactive oxygen species (ROS) are important signalling mediators and byproducts of cellular metabolism. A549 cells exhibit a high basal oxidative state due to KRAS-driven metabolic reprogramming (Weinberg et al., 2010). Moderate ROS levels promote proliferation via activation of MAPK and PI3K/AKT signaling, whereas excessive ROS can trigger mitochondrial dysfunction and apoptosis through cytochrome c release and caspase activation (Simon et al., 2000). Doxorubicin, cisplatin, and etoposide are examples of chemotherapy drugs that raise ROS levels in A549 cells, which aids in cytotoxicity. But long-term exposure frequently triggers adaptive antioxidant responses that are mediated by Nrf2 (nuclear factor erythroid 2-related factor 2), which increases the activity of detoxifying enzymes such as superoxide dismutase and glutathione S-transferases (Taguchi et al., 2011). This adaptive mechanism enables A549 cells to evade apoptosis and develop resistance to ROS-inducing therapies (DeNicola et al., 2011). Targeting ROS regulation, either by enhancing oxidative stress (pro-oxidant therapy) or inhibiting Nrf2-mediated antioxidant defenses, has been proposed as a therapeutic strategy in NSCLC, with A549 cells serving as a critical testing platform(Panieri and Santoro, 2016). The influence of ROS on drug response mechanisms in A549 cells is schematically illustrated in (Figure 3).

Drug resistance in A549 cells

Therapeutic resistance in A549 cells mirrors clinical patterns seen in NSCLC and arises from multifactorial mechanisms that span drug transport, stress-response signaling, tumor-cell plasticity, and cues from the tumor microenvironment. Together, these adaptations blunt cytotoxic and targeted therapies and promote disease persistence.

Mechanisms of Resistance

Efflux transporters and intracellular drug disposition

A central mechanism in A549 (and many NSCLC models) is ATP-binding cassette (ABC) transporter–mediated efflux, which lowers intracellular drug concentration. Overexpression/activation of ABCB1 (P-glycoprotein/MDR1), ABCC1 (MRP1), ABCC3 (MRP3), ABCC10 (MRP7), and ABCG2 (BCRP) has been associated with reduced sensitivity to anthracyclines, taxanes, and nucleoside analogs (Cole et al., 1992; Doyle et al., 1998; Gottesman et al., 2002; Hopper-Borge et al., 2004; Szakács et al., 2006). Of these, MRP1/3 are frequently detectable in lung adenocarcinoma lines, while MRP7 can confer resistance to taxanes and nucleoside

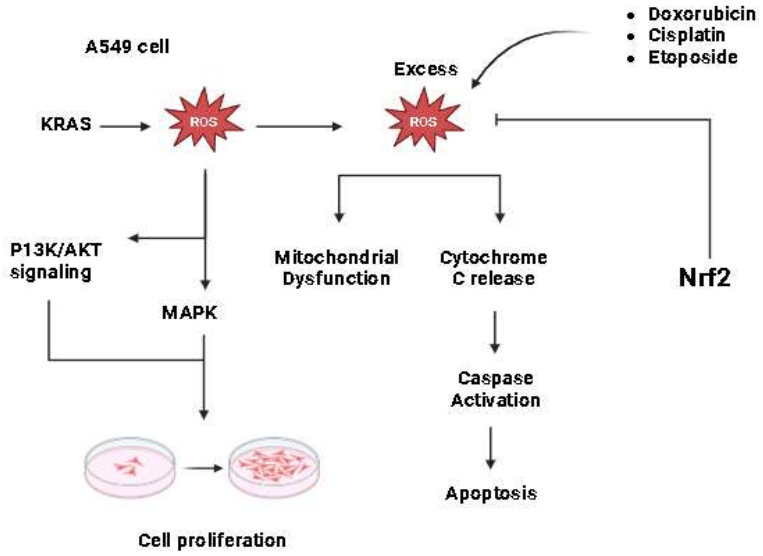

Figure 3: ROS-Mediated Pathway Driving Proliferation, Apoptosis, and Drug Response in A549 Cells (Created in: https://BioRender.com).

analogs through broad-substrate efflux. ABC transporter induction may be driven by NF-κB and AP-1 signaling or by exposure-induced selection of resistant subclones (Doyle et al., 1998; Gottesman et al., 2002; Holohan et al., 2013; Szakács et al., 2006).

Gene-expression reprogramming, EMT, and stress pathways

A549 cells display transcriptional plasticity under drug pressure, including upregulation of pro-survival signaling (PI3K/AKT, NF-κB), anti-apoptotic proteins (BCL-2 family), DNA-damage tolerance programs, and epithelial–mesenchymal transition (EMT) signatures that enhance motility, survival, and drug efflux (Bentires-Alj et al., 2003; Engelman, 2009; Hoesel and Schmid, 2013; Holohan et al., 2013; Karin, 2009; Lamouille et al., 2014; Vara et al., 2004; Vasan et al., 2019; W. Zhang et al., 2023). EMT is commonly triggered by TGF-β, inflammatory cytokines, or hypoxia, and is associated with resistance to multiple agents via crosstalk with AKT/ERK and NF-κB (Karin, 2009; Lamouille et al., 2014; Z. Zhang et al., 2012). In parallel, Nrf2 activation (often via KEAP1 dysregulation in lung adenocarcinoma) augments antioxidant and xenobiotic-detoxification enzymes (e.g., GSH synthesis, SOD, GSTs), conferring tolerance to ROS-inducing chemotherapies (DeNicola et al., 2011; Panieri and Santoro, 2016; Singh et al., 2006; Taguchi et al., 2011).

Microenvironmental factors: hypoxia, 3D architecture, and stromal interactions

Hypoxia stabilizes HIF-1α, reprogramming metabolism toward glycolysis, reducing drug uptake, and promoting quiescence and EMT—each dampening chemo- and radio-sensitivity (Brown and Wilson, 2004; Semenza, 2003). 3D spheroids/organoids of A549 create diffusion gradients (oxygen, nutrient, drug) and cell–ECM contacts that raise apoptotic thresholds and recapitulate multicellular resistance seen in tumors (Edmondson et al., 2014; Vinci et al., 2012). Cancer-associated fibroblasts (CAFs) and other stromal cells secrete paracrine factors (e.g., HGF, IL-6) or provide metabolites that bypass drug action and activate survival pathways (ERK, AKT, STAT3), fostering non-cell-autonomous resistance (Junttila and de Sauvage, 2013; Straussman et al., 2012; W. Zhang et al., 2023). Enrichment of cancer stem-like cells (CSCs)—including $CD90^{+}$ subpopulations reported in A549—further contributes to durable resistance and relapse (Phi et al., 2018; Yan et al., 2013).

Case Study: Gemcitabine Resistance in A549

Gemcitabine (dFdC) requires cellular uptake via equilibrative/ concentrative nucleoside transporters (notably hENT1/SLC29A1), intracellular activation by deoxycytidine kinase (dCK), and evasion of catabolism by cytidine deaminase (CDA). Resistance in A549 models typically involves:

(i) Reduced uptake (downregulation of hENT1);

(ii) Insufficient activation (loss/attenuation of dCK);

(iii) Enhanced catabolism (upregulated CDA); and

(iv) Increased DNA repair/synthesis capacity (elevated RRM1/RRM2), which counteracts gemcitabine-mediated ribonucleotide reductase inhibition (Bergman et al., 2002;

Giovannetti et al., 2005; Mini et al., 2006).

Experimentally selected gemcitabine-resistant A549 sublines show stable phenotypes with altered transporter/enzymatic profiles and survival signaling rewiring, supporting these mechanisms (Ikeda et al., 2011). Crosstalk with NF-κB and Nrf2 pathways further buffers gemcitabine-induced ROS and apoptosis, while EMT/CSC enrichment increases tolerance to nucleoside analogs (DeNicola et al., 2011; Karin, 2009; Lamouille et al., 2014; Mini et al., 2006; Panieri and Santoro, 2016; Taguchi et al., 2011; Z. Zhang et al., 2012).

Case Study: Adriamycin (Doxorubicin) Resistance in A549

Doxorubicin exerts cytotoxicity via DNA intercalation, topoisomerase II inhibition, and ROS generation. A549 resistance is commonly linked to:

(i) ABC transporter overexpression (notably ABCB1/MDR1 and ABCC1/MRP1) that lowers nuclear drug levels (Bentires-Alj et al., 2003; Cole et al., 1992; Doyle et al., 1998; Gottesman et al., 2002; Szakács et al., 2006);

(ii) Alterations in TOP2A levels or function, diminishing target engagement (Pommier et al., 2010);

(iii) Enhanced antioxidant defenses (Nrf2-driven), which mitigate doxorubicin-induced ROS and apoptosis (DeNicola et al., 2011; Panieri and Santoro, 2016; Singh et al., 2006; Taguchi et al., 2011; Trachootham et al., 2009); and

(iv) Pro-survival signaling (AKT/NF-κB) and apoptotic checkpoint remodeling (BCL-2, XIAP) (Bentires-Alj et al., 2003; Chen et al., 2024; Hoesel and Schmid, 2013; Holohan et al., 2013; Karin, 2009).

Cumulative effects yield a high apoptotic threshold and persistent clonogenic survival even at cytotoxic exposures.

Strategies to Overcome Resistance

Transporter modulation and pharmacologic bypass

MDR modulators (first/second-generation P-gp inhibitors such as verapamil or cyclosporine A, and later tariquidar) can restore intracellular drug levels and resensitize resistant cells in preclinical systems, though clinical translation is constrained by off-target toxicity and pharmacokinetic interactions (Dantzig et al., 1996; Fox and Bates, 2007; Lum et al., 1993). Alternative strategies include drug reformulation (liposomes, nanoparticles) to enhance tumor delivery and non-substrate analogs that evade efflux pumps (Holohan et al., 2013; Vasan et al., 2019).

Network-level co-targeting

Given crosstalk among PI3K/AKT, MEK/ERK, and NF-κB, dual-pathway inhibition has shown synergy in A549—e.g., PI3K/mTOR blockade combined with MEK inhibition—to surmount compensatory signaling and lower apoptotic thresholds (Faber et al., 2009; Hoesel and Schmid, 2013; Holohan et al., 2013; Qiang et al., 2025; Vasan et al., 2019). In ROS-buffered states, Nrf2 pathway inhibition (genetic or pharmacologic) can re-enable oxidative lethality from anthracyclines or platinum agents (DeNicola et al., 2011; Panieri and Santoro, 2016; Singh et al., 2006; Taguchi et al., 2011; Trachootham et al., 2009). Rational combinations should be guided by pathway activation status and toxicity windows.

Targeting EMT/CSC programs and the microenvironment

EMT reversal (TGF-β/AXL/FAK inhibitors) and CSC-directed approaches (e.g., NOTCH/Hedgehog blockade, metabolic targeting) reduce the pool of intrinsically tolerant cells and can delay resistance emergence (Lamouille et al., 2014; Phi et al., 2018; Z. Zhang et al., 2012). Microenvironment-informed regimens—including hypoxia-targeted therapies, stromal-signaling blockers (e.g., IL-6/JAK/STAT3), or 3D-optimized dosing—address diffusion barriers and paracrine survival signals that protect A549 spheroids or co-cultures (Brown and Wilson, 2004; Edmondson et al., 2014; Junttila and de Sauvage, 2013; Semenza, 2003; Straussman et al., 2012; Vinci et al., 2012). Finally, schedule optimization (sequential vs concurrent therapy) can exploit transient vulnerabilities created by pathway perturbations or redox imbalances (Holohan et al., 2013; Trachootham et al., 2009; Vasan et al., 2019).

Cancer stem cell dynamics in A549

In addition to the drug resistance pathways mentioned above, the existence of cancer stem cell (CSC)-like subpopulations in A549 cells is a significant factor in treatment failure. These cells make up a small portion of the total tumour cell population, but they exhibit traits including self-renewal, differentiation flexibility, and increased stress tolerance that allow them to continue growing tumours for a long time and cause relapse after treatment.

Identification of CSC Populations in A549

In A549 cultures, CSC-like cells have been detected through surface-marker expression and functional assays. Subsets enriched for CD90 (Thy-1), CD44, and CD133, as well as those with elevated aldehyde dehydrogenase (ALDH1) activity, demonstrate enhanced clonogenicity and tumor-initiating capacity compared with non-CSC counterparts (Bertolini et al., 2009; Eramo et al., 2008; Jiang et al., 2009). For instance, $CD90^+$ A549 cells generate tumors more efficiently in xenografts and show relative resistance to cisplatin and gemcitabine, whereas $CD90^-$ populations display limited tumorigenic potential (Yan et al., 2013). Similarly, ALDH1-high cells tolerate oxidative

stress and drug-induced cytotoxicity, highlighting metabolic resilience as a CSC hallmark (Jiang et al., 2009).

Role in Tumorigenesis and Relapse

The persistence of CSC-like cells in A549 not only sustains primary tumor growth but also fuels relapse after treatment. Conventional chemotherapy typically eliminates bulk tumor cells, yet CSC-enriched subpopulations survive due to Efficient DNA repair mechanisms, enabling recovery from drug- or radiation-induced damage (Bao et al., 2006). Overexpression of ABC transporters such as ABCG2, which limit intracellular drug accumulation (Dean et al., 2005). Adoption of quiescent or slow-cycling states, reducing vulnerability to agents that preferentially target proliferative cells (Moore and Lyle, 2011). This survival advantage allows CSCs to regenerate the tumor following therapy, recapitulating both heterogeneity and resistance. Clinical correlations reinforce this model, as CSC-associated markers (CD133, ALDH1) in lung tumors are linked to shorter progression-free survival and poorer overall outcomes (Bertolini et al., 2009; Sullivan et al., 2010).

Targeting CSC-Driven Resistance in A549

Given their role in perpetuating resistance, CSCs represent a priority therapeutic target in A549 models. Several strategies are under investigation: Pathway inhibition: Blockade of Notch, Wnt, or Hedgehog signaling, which sustains CSC self-renewal, reduces tumorigenicity, and sensitizes A549 cells to chemotherapy. Surface marker targeting: Interference with CD44–hyaluronan interactions or depletion of $CD90^{+}$ subsets can impair CSC maintenance (Misra et al., 2011). Metabolic disruption: CSCs exhibit metabolic flexibility, relying on both glycolysis and oxidative phosphorylation. Agents like metformin selectively reduce CSC viability in A549 models (Janzer et al., 2014). Epigenetic reprogramming: CSC drug tolerance is often reversible through chromatin remodeling; HDAC and DNMT inhibitors destabilize CSC states and restore chemosensitivity (Sharma et al., 2010). Combination therapies: The most effective approaches involve dual targeting—eliminating the tumor bulk with cytotoxic drugs while suppressing CSC self-renewal. For example, cisplatin combined with Notch inhibitors delays recurrence in A549 xenografts (Abel and Simeone, 2013).

Emerging therapeutic strategies using A549 models

New directions for investigating therapeutic approaches in NSCLC have been made possible by developments in biotechnology and computational sciences. Advanced technologies have been widely tested and validated using A549 cells, a well-characterized adenocarcinoma model. Among these, 3D culture/organoid models, CRISPR-Cas9 gene editing, and artificial intelligence (AI) integration are revolutionary techniques that connect fundamental research with translational medicine.

CRISPR-Cas9 for Gene Editing

Mapping genetic vulnerabilities

The advent of CRISPR-Cas9 genome editing has enabled precise dissection of gene function in A549 cells, offering unprecedented opportunities to map oncogenic drivers, synthetic lethal partners, and drug-resistance determinants (Shalem et al., 2015). Genome-scale CRISPR knockout and CRISPR interference (CRISPRi) libraries applied to A549 have revealed dependencies on essential pathways such as KRAS signaling, DNA damage repair (ATM, BRCA1/2), and antioxidant responses (Nrf2/KEAP1) (Wang et al., 2024). These insights have illuminated potential vulnerabilities that can be therapeutically exploited, such as sensitization to PARP inhibitors in DNA repair-deficient contexts (McCarthy, 2005).

Functional genomics applications

Functional CRISPR screens in A549 cells have also been instrumental in uncovering genes that mediate immune evasion (e.g., regulators of PD-L1 expression) and drug resistance (e.g., loss of pro-apoptotic mediators or upregulation of efflux transporters) (Manguso et al., 2017). Importantly, CRISPR allows rapid generation of isogenic A549 sublines carrying specific mutations (e.g., KRAS G12C vs. G12S) for comparative drug testing, thus modeling patient-specific responses (Kim et al., 2016). Such applications place A549 at the forefront of CRISPR-driven functional genomics.

3D Cultures and Organoids

Advantages over 2D models

Conventional 2D monolayer cultures of A549 cells provide ease of use but lack the spatial, architectural, and microenvironmental complexity of human tumors. Transitioning to 3D culture systems—including spheroids and organoids—offers a more physiologically relevant model, with gradients of oxygen, nutrients, and drugs that mimic in vivo tumors (Edmondson et al., 2014). These systems allow the study of cell–cell interactions, hypoxia-driven resistance, and ECM remodeling, phenomena that cannot be adequately captured in 2D.

Applications in personalized medicine

A549-derived organoid models can be engineered to recapitulate patient-specific tumor mutations and tested against therapeutic panels, supporting precision oncology approaches. For example, 3D A549 organoids exposed to immune checkpoint inhibitors or KRAS-targeted agents exhibit treatment responses more predictive of in vivo behavior compared with 2D cultures (Neal et al., 2018).

Moreover, co-culture of A549 organoids with stromal or immune cells enables modeling of microenvironment interactions, further enhancing translational relevance (Esteva et al., 2019).

Integration with Artificial Intelligence

AI in drug screening and predictive modeling

Artificial intelligence (AI) has emerged as a powerful complement to A549-based studies. Machine learning algorithms can analyze large-scale omics datasets generated from CRISPR screens, transcriptomics, or proteomics in A549 cells, enabling identification of biomarkers and predictive signatures of therapy response (Esteva et al., 2019). AI-driven predictive modeling also accelerates virtual drug screening, allowing prioritization of compounds before wet-lab validation in A549 assays (Zhavoronkov et al., 2019).

Synergy with CRISPR and organoid systems

The integration of AI with CRISPR-Cas9 and 3D organoid platforms is particularly promising. For example, AI can help design optimized sgRNA libraries for CRISPR functional genomics in A549, minimizing off-target effects while maximizing coverage (Doench et al., 2016). Similarly, data from high-throughput drug testing on A549 organoids can be fed into AI models to predict patient-specific treatment outcomes, bridging experimental systems with clinical decision-making (Jiao et al., 2020).

Critical evaluation of A549 as a model

The A549 cell line has become one of the most widely utilized in vitro systems for studying NSCLC, particularly adenocarcinoma. Its extensive use in cancer biology, drug discovery, and molecular pathway analysis reflects both its strengths as a reproducible model and its limitations in capturing the full complexity of lung cancer biology. A balanced appraisal is therefore essential to contextualize findings derived from A549 studies.

Strengths of the A549 Model

One of the primary advantages of A549 cells is their reproducibility and stability in culture, which enables consistent experimental outcomes across laboratories worldwide (Gazdar et al., 2010). The well-documented genetic background of A549, including KRAS mutations and TP53 alterations, provides relevance to common oncogenic pathways in NSCLC (Imielinski et al., 2012). This genetic landscape allows mechanistic insights into tumor signaling, metabolism, and drug resistance that mirror clinical phenotypes (Singh and Settleman, 2010).

Additionally, A549 cells display epithelial morphology, surfactant production, and expression of alveolar type II pneumocyte markers, which provide a biologically meaningful context for modeling lung adenocarcinoma (Lieber et al., 1976). Their adaptability to 2D and 3D cultures, as well as compatibility with CRISPR-based gene editing, further enhances their utility as a versatile platform for preclinical investigations (Meyers et al., 2017).

Limitations of A549 as a Model

Despite these strengths, important limitations restrict the translational accuracy of A549 findings. A critical drawback is the absence of ciliated epithelial features and the inability to fully represent the cellular diversity of the lung epithelium (Rock et al., 2010). Furthermore, A549 monolayers lack the tumor microenvironment (TME)—including stromal fibroblasts, immune cells, and vascular components—that profoundly influence tumor growth and therapeutic responses (Hanahan and Coussens, 2012).

A further limitation is the clonal evolution and prolonged adaptation of A549 cells in culture, which can cause them to deviate from the characteristics of the original tumor (Ben-David et al., 2018). This adaptation can lead to discrepancies between preclinical drug responses in A549 and patient outcomes. Moreover, the predominance of KRAS mutations in A549 makes it less representative of EGFR-driven NSCLC, which is highly prevalent in Asian populations (Mok et al., 2009).

Need for Integration with Complementary Models

Given these limitations, A549 should be viewed as part of a complementary toolkit rather than a stand-alone model. Incorporating patient-derived xenografts (PDXs), genetically engineered mouse models (GEMMs), and patient-derived organoids is essential to reflect the heterogeneity and intricate biology of NSCLC. Co-culture approaches that include stromal and immune components can further improve the translational fidelity of A549-based assays (Neal et al., 2018).

Future research should emphasize the strategic use of A549 alongside complementary systems, leveraging its reproducibility and pathway relevance while mitigating its limitations in microenvironmental representation. Such integrated approaches are more likely to yield preclinical findings that translate into effective therapies for NSCLC patients.

Future perspectives

The evolution of A549 research reflects the dynamic intersection of molecular biology, biotechnology, and computational science in advancing NSCLC models. While A549 cells have yielded invaluable insights into cancer signaling, drug resistance, and therapeutic response, the next phase of research will require integrative and patient-

tailored approaches that address the inherent limitations of 2D cell lines. Emerging innovations—particularly patient-derived organoid systems, AI-driven analytics, and CRISPR-engineered 3D models—offer opportunities to transform A549 from a conventional tool into a cornerstone of next-generation translational oncology.

Patient-Derived A549 Organoid Systems

Future efforts are likely to focus on deriving organoid systems from A549 cells that incorporate patient-specific genetic profiles and microenvironmental cues. By embedding A549-derived organoids in extracellular matrix scaffolds or co-culturing them with stromal and immune cells, researchers can better replicate the tumor microenvironment and heterogeneity (Sachs and Clevers, 2014). These organoids provide valuable platforms for drug screening, biomarker validation, and immunotherapy research, thus connecting basic in vitro models with more sophisticated in vivo systems.

AI-Driven Precision Oncology Pipelines

The increasing availability of multi-omics datasets from A549—including genomics, transcriptomics, proteomics, and metabolomics—provides fertile ground for artificial intelligence (AI)-driven predictive modeling. Future pipelines could integrate these datasets to forecast drug responses, identify resistance trajectories, and stratify patients for individualized therapies (Kuenzi et al., 2020). Such approaches may also support adaptive clinical trial design, where A549-derived data guide therapeutic decision-making in real time. Importantly, AI holds potential to synergize with organoid pharmacotyping, producing predictive models that are more representative of patient outcomes.

CRISPR + 3D Models for Next-Generation NSCLC Research

The combination of CRISPR-Cas9 gene editing with 3D/organoid cultures is poised to become a transformative strategy in NSCLC research. A549-derived 3D systems edited via CRISPR allow the generation of isogenic organoid panels carrying clinically relevant mutations (e.g., KRAS G12C vs. G12S, TP53 variants), which can then be subjected to drug sensitivity assays (Tsherniak et al., 2017). These next-generation models will facilitate synthetic lethality mapping, evaluation of immune checkpoint regulation, and testing of nanomedicine delivery platforms under physiologically relevant conditions (Antoni et al., 2015).

Conclusion

The A549 cell line has been a pivotal tool in NSCLC investigations for nearly half a century, delivering a stable and genetically representative platform for exploring lung adenocarcinoma mechanisms. Using this model, researchers have elucidated crucial aspects of molecular pathways, drug resistance, and cancer stem cell dynamics, facilitating progress in conventional as well as emerging treatment strategies. Its adaptability to 2D and 3D cultures, compatibility with CRISPR-Cas9 gene editing, and role in evaluating immunotherapies, nanomedicine, and AI-driven approaches underscore its enduring relevance in translational oncology. Yet, despite its strengths, the A549 model has inherent limitations, including the absence of ciliated epithelial features, incomplete representation of the tumor microenvironment, and culture-induced divergence from primary tumor biology. These limitations call for careful interpretation of results and emphasize the importance of integrating complementary models—such as patient-derived organoids, xenografts, and co-culture systems—that more accurately capture tumor heterogeneity. Looking forward, the future of A549 research lies not in its replacement but in its strategic reinvention. By embedding A549 studies within multi-platform pipelines—encompassing patient-derived organoid systems, CRISPR-engineered models, and AI-based analytics—researchers can generate insights that are both mechanistically rigorous and clinically relevant. In this way, A549 will continue to serve as a bridge between molecular biology and precision oncology, contributing to the discovery of more effective therapies for patients with NSCLC.

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Conflict of interest

The authors declare that they have no known financial or personal conflicts of interest that could have appeared to influence the work reported in this paper.

Use of artificial intelligence declaration

Authors declare that artificial intelligence tools were used solely for grammar checking and language refinement of the manuscript. No AI tools were used for content generation, data analysis, interpretation of results, or

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