cancers

MDPI

Review

The Potential of the ISG15–Cytoskeletal Axis in Cancer Progression and Therapy Resistance

Shreya Pillai [1,†], Ngan Vo [1,†], Navnath S. Gavande [2,3] and Dipankar Ray [1,*]

[1] Department of Radiation Oncology, University of Michigan, Ann Arbor, MI 48109, USA; shreyapi@umich.edu (S.P.); nganvo@umich.edu (N.V.)
[2] Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI 48201, USA; ngavande@wayne.edu
[3] Molecular Therapeutics Program, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA
* Correspondence: dipray@umich.edu
† These authors contributed equally to this work.

Simple Summary

Resistance to chemotherapy, radiation, and immunotherapy remains a major challenge in cancer treatment. Remodeling of the cytoskeleton, the internal framework that supports cell shape and movement, may help tumor cells survive treatment and spread. This review examines how interferon-stimulated gene 15 (ISG15), a protein involved in immune defense, may link cytoskeletal changes to treatment resistance. This relationship remains poorly understood, particularly because ISG15 can promote resistance in some cancers but improve treatment sensitivity in others. The review aims to synthesize existing evidence, identify key protein interactions, and highlight research gaps requiring experimental validation. By distinguishing established mechanisms from proposed connections, it can help guide researchers toward studies that test whether these interactions directly cause resistance. These insights may reveal potential new therapeutic targets and markers for predicting treatment response, supporting more personalized cancer care. Further laboratory and clinical studies are needed to establish their safety, effectiveness, and future clinical value.

Abstract

Interferon-stimulated gene 15 (ISG15) is a context-dependent regulator of cancer progression and therapeutic response, with both pro-tumor and anti-tumor effects reported across different cancer models. Accumulating evidence from our group and others suggests that ISG15 influences cytoskeletal organization, receptor trafficking, DNA damage repair, immune checkpoint regulation, and oncogenic signaling. However, the mechanisms linking ISG15 and cytoskeletal remodeling are poorly understood, and it is unclear whether this relationship is direct or associative. Given the role of cytoskeletal remodeling in processes such as cellular adaptation, elucidating how ISG15 influences these processes may provide important insights into ISG15's effects on tumor cell behavior. Here, we review the emerging relationship between ISG15 and cytoskeletal remodeling to examine potential mechanisms through which ISG15 may contribute to cancer progression and therapeutic resistance. We also discuss potential ISG15-associated cytoskeletal substrates, emerging therapeutic strategies, and translational challenges associated with targeting the ISG15–cytoskeletal axis. Our discussions highlight the need for further studies to establish the ISG15–cytoskeletal relationships and determine how the context-dependent effects of ISG15 shape tumor progression and therapeutic response.

Check for updates

Academic Editor: Alain P. Gobert

Received: 2 October 2026
Revised: 13 November 2026
Accepted: 23 November 2026
Published: 26 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Keywords: ISG15; ISGylation; cytoskeleton; therapy resistance; EMT; PD-L1; actin remodeling

1. Introduction

Therapy resistance remains a major challenge in the treatment of solid tumors which frequently develop mechanisms to evade chemotherapy, radiation therapy, and immunotherapy [1]. Emerging evidence suggests that many of these resistance pathways are linked to cytoskeletal reprogramming, a process that provides tumor cells with both mechanical adaptability and enhanced survival signaling [2–5].

One of the most prominent cytoskeleton-associated resistance mechanisms is epithelial–mesenchymal transition, or EMT [6,7]. EMT increases tumor cell plasticity, migratory capacity, and invasiveness while also impairing anti-tumor immunity and reducing sensitivity to chemotherapy and radiation [7]. Cytoskeletal proteins, including actin microfilaments and their regulatory proteins, microtubules and microtubule-associated proteins, and intermediate filaments, are central to this transition [6]. Their remodeling alters cell polarity, intracellular trafficking, mechanotransduction, and stress responses, ultimately supporting cancer progression, metastasis, and therapeutic resistance [6,8].

A growing body of evidence implicates interferon-stimulated gene 15 (ISG15) as an important regulator of cytoskeletal reorganization and therapy resistance [9]. ISG15 is a type I interferon-responsive ubiquitin-like protein best known for its antiviral function [10]. Through ISGylation, ISG15 covalently modifies target proteins to limit viral replication and enhance host defense [10]. ISG15 can function in both free and conjugated forms, modifying or interacting with diverse substrates, including cytoskeletal and signaling proteins [9]. Dysregulated ISG15 expression has been implicated in oncogenesis and treatment response across multiple cancer types [11–13]. ISG15 may influence EMT, immune evasion, and survival under therapeutic stress [6,7,9,12,14,15].

This review proposes that ISG15 represents a mechanistic link between cytoskeletal remodeling and therapy resistance. We first summarize the major cytoskeletal systems involved in cancer biology, then discuss how ISG15 regulates cytoskeletal architecture and resistant phenotypes. Finally, we evaluate therapeutic and translational opportunities associated with targeting ISG15, ISGylation, and ISG15-associated cytoskeletal substrates.

2. Cytoskeletal Dynamics in Cancer

2.1. Cytoskeletal Proteins and Their Roles

The cellular cytoskeleton is a complex, highly dynamic network composed of three major filament systems: microfilaments, microtubules, and intermediate filaments. Together, these structures regulate cell shape, biomechanics, motility, intracellular transport, and cell division. The cytoskeleton continuously reorganizes in response to internal and external signals, enabling essential processes such as migration, force transmission, endocytosis, and mitosis [16]. Although each filament class has distinct structural and functional properties, all three systems work in coordination with associated regulatory proteins to provide mechanical support, organize intracellular architecture, and orchestrate mechanotransduction, cell division, migration, and maintenance of tissue integrity [16].

Microfilaments, also known as actin filaments, are composed of polymerized globular actin monomers that form polarized filamentous actin structures. These filaments generate forces required for focal adhesion formation, cell movement, and dynamic changes in cell shape. Actin organization is tightly regulated by numerous accessory proteins, including the Arp2/3 complex, formins, cofilin, and cross-linking proteins such as α-actinin and filamin,

which control filament nucleation, elongation, branching, severing, and bundling [16–19]. Through coordinated interactions, the actin cytoskeleton establishes cell polarity, mechanical integrity, and motility. Microtubules are another essential component of the cytoskeleton. Composed of α- and β-tubulin heterodimers, microtubules organize intracellular trafficking, support organelle positioning, and mediate mitotic spindle formation [20]. The third major class of cytoskeletal proteins is intermediate filaments, or IFs, which provide cells with mechanical resilience and long-term structural stability. Unlike actin filaments and microtubules, which are highly dynamic, IFs are generally more stable and tensile, allowing cells to withstand shear stress, stretching, and deformation without rupture [21]. IFs are also highly tissue-specific, reflecting their specialized roles across different cell types [21].

Importantly, these cytoskeletal systems do not function in isolation. Instead, actin filaments, microtubules, and intermediate filaments form an integrated network that coordinates cell shape, polarity, intracellular trafficking, and mechanical signaling. Through these interactions, cytoskeletal proteins contribute not only to structural integrity but also to dynamic processes such as migration, stress adaptation, signaling reprogramming, and survival. In cancer, alterations in cytoskeletal proteins reflect broader functional reprogramming, as cytoskeletal remodeling enhances cellular plasticity, motility, invasiveness, and resistance to therapy.

2.2. The Role of Cytoskeletal Proteins in Cancer Progression

Cytoskeletal remodeling is a defining feature of cancer progression. As epithelial cells acquire invasive and metastatic properties, they undergo profound structural and functional changes governed largely by the actin cytoskeleton, microtubules, and intermediate filaments. These alterations are central to EMT, a process through which tumor cells lose epithelial polarity and adhesion, reorganize their cytoskeletal architecture, and adopt a more plastic and motile phenotype. In addition to promoting invasion and metastasis, cytoskeletal reprogramming contributes significantly to therapeutic resistance [22].

2.2.1. Actin Cytoskeleton Remodeling and Tumor Invasion

Altered activity of actin-modulating proteins can enhance tumor cell invasiveness and drive structural remodeling associated with EMT [23]. Early primary culture studies examining the progression of human colon epithelial lesions revealed distinct changes in actin organization across tumor stages. Normal epithelium displays well-organized actin cables, preneoplastic lesions show reduced actin organization, and malignant carcinomas often lose actin cables entirely. These architectural transitions reflect the mechanical softening and increased motility required for invasive progression [24,25].

Actin-based structures, including lamellipodia, filopodia, stress fibers, and invadopodia, play essential roles in cancer cell migration, adhesion, and extracellular matrix degradation [26]. These protrusive and contractile structures depend on tightly regulated actin polymerization, branching, and bundling, coordinated by nucleators such as Arp2/3 and formins, as well as bundling proteins including fascin and tropomyosins [3,27].

Among these regulators, Fascin-1 is particularly important. As an actin-bundling protein that organizes filopodia and invadopodia, Fascin-1 is strongly upregulated in colorectal and gastric cancers, where it supports directional migration and correlates with worse clinical outcomes [28]. Similarly, transgelins, including TAGLN and TAGLN2, contribute to actin stabilization and contractility [29]. Altered expression of these proteins facilitates enhanced motility and supports the cytoskeletal remodeling characteristic of EMT.

Rho-family GTPases, including Rho, Rac, and Cdc42, serve as master regulators of actin remodeling and are closely involved in EMT. Rho promotes stress fiber and focal adhesion formation, Rac drives lamellipodia formation, and Cdc42 regulates filopodia [30,31].

Functional inhibition of these GTPases in model systems markedly reduces lamellipodia and stress fiber formation, impairs adhesion, and suppresses cell spreading [32]. Dysregulated Ras-Rho signaling is common across many tumor types, providing a mechanistic link between oncogenic signaling pathways and cytoskeletal transitions that support EMT, invasion, and metastasis.

2.2.2. Microtubule Remodeling and the "Tubulin Code" in Cancer

Microtubules undergo numerous post-translational modifications, or PTMs, including acetylation, detyrosination, and glutamylation. Collectively, these modifications constitute the "tubulin code," which fine-tunes microtubule stability, intracellular trafficking, and cell polarity during migration. In cancer cells, altered tubulin PTMs contribute to EMT, metastasis, and chemoresistance by reshaping microtubule dynamics and enhancing cell survival under stress [33].

Microtubule dynamics also support the trafficking of receptors, integrins, and cytoskeletal regulators, thereby coordinating actin-microtubule crosstalk required for efficient migration [34,35]. Given these diverse functions, microtubule dysregulation contributes to key aspects of metastatic behavior and resistance to microtubule-targeting chemotherapeutics.

2.2.3. Intermediate Filaments: Vimentin and Lamin-Dependent Mechanics

Intermediate filaments provide mechanical resilience and structural support, and among them, vimentin is a hallmark of EMT. In cancer cells, vimentin upregulation is associated with increased motility, cytoskeletal plasticity, and mesenchymal morphology. Its expression frequently correlates with poor prognosis and aggressive disease. Beyond shaping cell mechanics, vimentin influences organelle positioning, intracellular signaling, and interactions with actin filaments and microtubules [36].

Coexpression of vimentin with epithelial keratins reflects a hybrid EMT phenotype associated with increased stemness and greater metastatic potential [22]. This hybrid state allows tumor cells to retain certain epithelial traits while acquiring mesenchymal features that support invasion, survival, and dissemination.

Within the nucleus, lamins regulate nuclear stiffness, shape, and mechanotransduction through the LINC complex. Altered lamin expression during EMT promotes nuclear deformability, allowing cancer cells to migrate through confined tissue spaces. These changes also influence gene expression, DNA damage responses, and cellular adaptation to mechanical stress [37]. Together, these nuclear–cytoskeletal interactions enhance the invasive and adaptive capabilities of tumor cells.

Overall, cytoskeletal remodeling in cancer involves coordinated changes in actin dynamics, microtubule PTMs, intermediate filament expression, and nuclear-mechanical coupling. These transitions provide mechanical flexibility, motility, and signaling adaptability required for EMT, invasion, metastasis, and survival under therapeutic stress.

3. ISG15 as a Regulator of Therapeutic Sensitivity and Resistance

3.1. ISG15 in Therapeutic Sensitivity and Resistance

Growing evidence links ISG15 to altered responses to cancer therapeutics. Although ISG15 is induced as part of the host antiviral defense response [10], it is also upregulated in several cancers, where it can contribute to immune evasion and tumor progression. Accordingly, ISG15 has been implicated in modulating cancer cell responses to chemotherapy, radiotherapy, and immunotherapy [38–45].

In esophageal adenocarcinoma (EAC), ISG15 is highly upregulated and contributes to T-cell impairment. A majority of EAC patients exhibit poor response rates to standard

chemoradiation, and elevated tumor ISG15 expression is associated with worse prognosis [38]. Mechanistically, ISG15 is upregulated following ionizing radiation and promotes the recruitment of homologous recombination repair proteins, particularly γH2AX, to DNA damage sites, thereby contributing to radioresistance in EAC [39].

The association between ISG15 and treatment resistance extends to other malignancies. In pancreatic cancer, ISG15 is overexpressed in high-grade tumors and is linked to poor clinical outcomes and gemcitabine resistance. In vitro, silencing ISG15 in resistant pancreatic cancer cell lines restores chemotherapy sensitivity [40]. In gastric cancer, ISG15 upregulation has been linked to the actin-binding protein TAGLN2, whose elevated expression promotes chemoradioresistance [41]. Higher ISG15 levels have also been observed in irinotecan-resistant tumors compared with treatment-sensitive counterparts [42].

ISG15 is also a core component of the interferon-related DNA damage resistance signature, or IRDS, a gene subset associated with resistance to chemotherapy and radiotherapy. Elevated ISG15 expression marks IRDS-positive tumors, and in breast cancer, IRDS-positive patients derive minimal benefit from chemotherapy compared with IRDS-negative patients [43].

However, ISG15 functions in a context-dependent manner, and in certain tumor types it can promote chemosensitivity rather than resistance. In several epithelial cancers, including pharyngeal squamous cell carcinoma, breast carcinoma, and head and neck squamous carcinoma, increased ISG15 levels are associated with improved responses to chemotherapeutic agents such as doxorubicin and camptothecin [44,45]. Similar observations have been reported in non-small cell lung cancer, or NSCLC, where higher ISG15 expression is associated with increased sensitivity to topoisomerase I inhibitors, whereas lower ISG15 expression is associated with resistance [46]. In ovarian cancer, reduced ISG15 expression is associated with cisplatin resistance and poorer outcomes, while restoring ISG15 expression enhances chemosensitivity [47,48]. Likewise, in acute promyelocytic leukemia, ISG15 contributes to the myeloid differentiation response to all-trans retinoic acid [49].

Together, these findings indicate that ISG15 can modulate therapeutic response in cancer, although its effects vary by tumor type, treatment context, and molecular environment. One potential mechanism through which ISG15 influences therapy response is cytoskeletal remodeling, a process that shapes tumor cell morphology, plasticity, invasion, and survival.

3.2. Mechanisms Linking ISG15 to Cytoskeletal Regulation

ISG15 and ISGylation are increasingly recognized as regulators of cytoskeletal architecture and cellular plasticity. Rather than acting through a single mechanism, ISG15 influences multiple cellular systems, including direct modification of cytoskeletal and motor proteins, modulation of small GTPase signaling, regulation of receptor trafficking, and stabilization of oncogenic signaling pathways. ISGylation may also connect cytoskeletal machinery to DNA damage repair processes. Through these diverse functions, ISG15 may promote EMT and contribute to therapeutic resistance.

3.2.1. Direct Interactions Between ISG15 and Cytoskeletal Proteins

ISG15 can regulate cytoskeletal organization through direct interactions with structural and motor proteins. One example is its regulation of actomyosin contractility in breast cancer cells. Non-muscle myosin IIA, or NMIIA, is a key cytoskeletal motor protein involved in cell shape, contractility, migration, and adhesion. Studies in several breast cancer cell lines have shown that ISG15 directly interacts with NMIIA through ISGylation. ISG15-NMIIA colocalization and association occur during cell spreading, altering NMIIA interactions with other components of the actomyosin complex. These changes promote elongated

cell morphology and increased stress fiber formation, thereby influencing breast cancer progression and migration [50].

3.2.2. ISG15's Role in Replication Stress and DNA Damage Response

Based on multiple reports, ISG15's role in controlling replication stress has been well documented. One example is PCNA (Proliferating Cell Nuclear Antigen), which plays a critical role in maintaining genome stability. PCNA undergoes ISGylation, an important step in regulating the timely termination of translesion synthesis. It's possible that dysregulation of this interaction, such as ISG15 overexpression, could allow cancer cells to sustain damage-tolerant replication for longer, giving tumors a survival mechanism that allows damaged cells to continue replicating. Understanding whether and how ISG15 regulates PCNA function in cancer cells could uncover the balance between mutagenic replication, genome instability, and drug resistance, pointing toward a potential therapeutic strategy to resensitize tumors to DNA-damaging treatments [51]. Additionally, ISG15 is non-covalently associated with RECQ1 to stimulate its helicase activity to promote the restart of stalled replication forks. Furthermore, it has been reported that inactivation of the MRE11 nuclease complex, which causes replication stress, can induce ISG15 expression, enabling ISG15/ISGylation at stalled forks to stabilize replication machinery and prevent DNA collapse [52].

ISG15 may also regulate the DNA damage response (DDR) through mechanisms linked to cytoskeletal dynamics. Our recent study [39] demonstrated that ionizing radiation induces ISGylation of γH2AX, which promotes MDC1 retention and RAD51 recruitment at DNA damage sites. This facilitates homologous recombination repair in EAC cells. In parallel, γH2AX signaling recruits nuclear myosin I and V, which translocate DNA double-strand breaks, or DSBs, along nuclear actin filaments to the nuclear periphery. This process enables DSB clustering and end resection required for homologous recombination repair. A summary of such findings is shown in Figure 1. Given the established roles of ISG15 in both cytoskeletal regulation and DDR signaling [9,14,15,52], ISG15 may influence cytoskeletal organization involved in DNA repair. Future studies examining how ISG15 influences cytoskeletal proteins in the context of DDR could provide important insight into the mechanisms underlying therapeutic resistance in EAC and other cancers.

3.2.3. ISG15 Modulates Rho GTPase Signaling

ISG15 can also influence cytoskeletal dynamics indirectly by regulating GTPase signaling pathways that control cell shape and motility. Rho-family GTPases act as molecular switches that coordinate actin remodeling, adhesion, and migration. These proteins cycle between inactive GDP-bound and active GTP-bound states under the control of guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and Rho GDP-dissociation inhibitors (RhoGDIs). Together, these regulators enable spatially localized control of cytoskeletal remodeling [53]. Microtubules also interact with Rho GTPases to regulate cytoskeletal organization and metastatic behavior [35]. Among these GTPases, Rac1 is particularly important because it drives membrane protrusion and cell migration [53].

In oral squamous cell carcinoma (OSCC), elevated ISG15 expression is associated with increased metastasis and worse patient outcomes. In this model, ISG15 interacts with Rac1, preferentially binding the inactive Rac1-GDP form and displacing its inhibitory partner, RhoGDI. This promotes Rac1 activation, increases Rac1-GTP levels, and drives cytoskeletal reorganization and cell migration. These findings provide one possible mechanistic basis for the aggressive and metastatic phenotype observed in ISG15-high tumors [53]. Consistently, ISG15 knockdown in OSCC cells reduces lymph node metastasis and invasive behavior [53].

Elevated ISG15 expression has also been associated with poorer prognosis in breast cancer patients with lymph node metastasis [54].

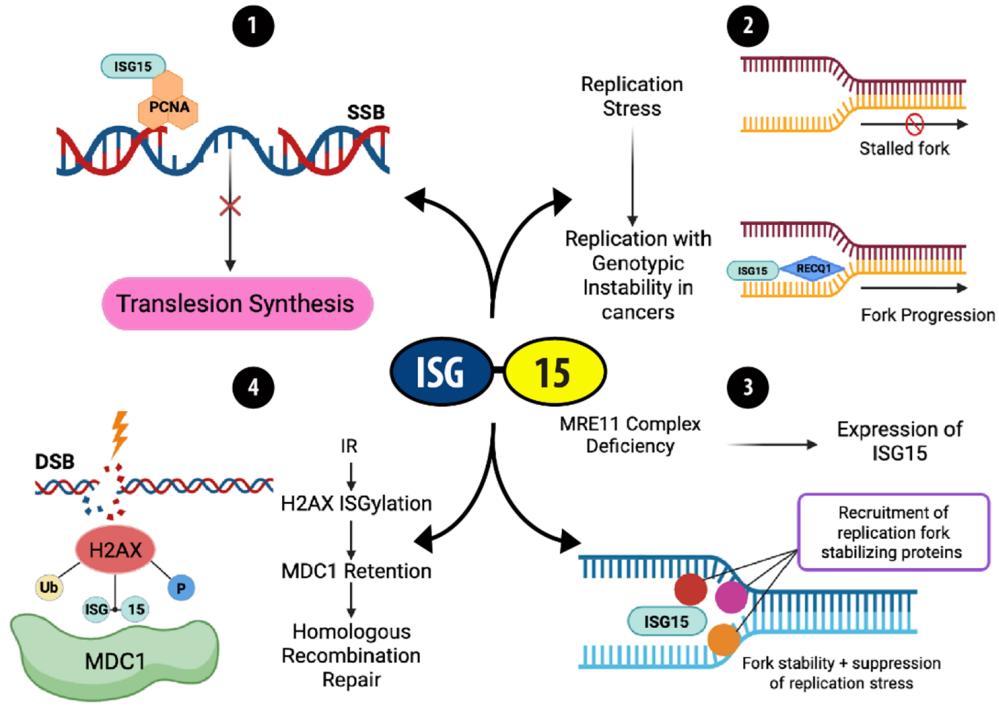

Figure 1. Multi-faceted roles of ISG15/ISGylation in controlling replication stress and DNA damage response. (**1**) Study shows PCNA as a substrate of ISGylation, which is important in regulating translesion synthesis to overcome replication stress. (**2**) Besides ISGylation, free ISG15 can interact and influence RECQ1, a helicase, to control replication fork movement, thus protects nascent DNA from degradation to reduce replication stress. (**3**) Following Mre11 complex inactivation, ISG15 is reported to be induced, which upon conjugation (ISGylation) to constituents of replication fork proteins (targets unclear) can prevent replication stress. (**4**) We recently reported that ionizing radiation induces DNA double-strand breaks (DSBs) and promotes ISGylation of γH2AX in esophageal adenocarcinoma (EAC) cells. ISGylated γH2AX enhances retention of MDC1 at DNA damage foci and facilitates RAD51 recruitment, thereby promoting homologous recombination repair. Created in BioRender. (https://app.biorender.com/illustrations/688104fae11fbd9b9425baf0). Further modifications were made in Adobe Illustrator 2026.

Additional evidence supports the role of ISG15 in regulating Rac1 activity. ISGylation targets modulators of Rac1 signaling, including DOCK6, a GEF that activates Rac1, and IQGAP1, a scaffold protein that stabilizes active Rac1-GTP. IQGAP1 undergoes extensive ISGylation, which weakens its interaction with Rac1 and constrains Rac1 signaling under conditions of elevated ISG15. In this context, ISG15 can function as a negative regulator of Rac1 activity. Consistent with this model, DOCK6 knockdown in HeLa cells triggered a compensatory reduction in ISG15 expression and IQGAP1 ISGylation, partially restoring Rac1 activity and enhancing migratory capacity. These findings highlight how ISG15 may fine-tune Rho GTPase signaling to regulate cytoskeletal plasticity [55].

3.2.4. ISG15 Regulates Membrane Trafficking and Oncogenic Signaling

ISG15 has also been reported to regulate Rab GTPases, another class of small GTP-binding proteins. Rab proteins control endosomal trafficking by recruiting effector molecules such as motor proteins and kinases. In cancer, Rab GTPases contribute to tumor progression through roles in cell migration, autophagy, and drug resistance [56]. In breast cancer models, ISG15 regulates Rab-dependent endocytic trafficking through ISGylation of Rab GDP-

dissociation inhibitor beta, or GDI2, which controls Rab localization and activity. ISGylation of GDI2 reduces its ability to bind Rab proteins such as Rab5, favoring EGFR recycling to the plasma membrane rather than degradation. This shift in receptor trafficking prolongs EGFR signaling and promotes sustained PI3K/Akt activation, enhancing proliferation and motility of breast cancer cells [54].

ISG15 also influences the Ras family of GTPases, including oncogenic KRAS. In breast cancer cells, ISGylation interferes with normal KRAS turnover, leading to KRAS stabilization and KRAS-driven disruption of cellular architecture. Silencing ISG15 restores cytoskeletal organization, as shown by increased actin polymerization, enhanced stress fiber formation, more spread cell morphology, and elevated E-cadherin expression, consistent with increased cell–cell adhesion and reduced migratory capacity. This study demonstrated that KRAS can induce ISG15 expression, while ISGylation limits lysosomal degradation of KRAS, forming a positive feedback loop that sustains KRAS stability [57].

Through these diverse functions, ISG15 can influence protein stability, cytoskeletal organization, receptor trafficking, and oncogenic signaling, thereby shaping tumor sensitivity to treatment. Although direct evidence linking ISG15-mediated cytoskeletal reprogramming to therapeutic resistance within the same experimental systems remains limited, accumulating data suggest that ISG15-driven changes, including actomyosin reorganization, Rac1-mediated plasticity, altered receptor recycling, and KRAS-dependent structural remodeling, promote morphological and signaling states associated with resistance. Table 1 summarizes prominent ISG15 targets that influence different cytoskeletal architectures driving cancer progression and therapy resistance.

Table 1. ISG15–Cytoskeletal Regulation in Cancer.

ISG15 Target	ISG15-Associated Mechanism	Expected Outcome	Cancer	Source
NMIIA (non-muscle myosin IIA)	ISGylation alters actomyosin complex organization	Stress fiber formation, elongated morphology, enhanced migration	Breast cancer	Cruz-Ramos, E. [50]
γH2AX	ISGylation of γH2AX promotes MDC1 retention and RAD51 recruitment at DSBs	Enhanced homologous recombination repair	EAC	Ghosh, A. [39]
Rac1	ISG15 binds Rac1-GDP, displaces RhoGDI, promotes Rac1 activation	Actin remodeling, migration, metastasis	OSCC	Chen, Y.L. [53]
DOCK6/IQGAP1	ISGylation of IQGAP1 weakens Rac1 interaction	Fine-tuning of migratory plasticity	Cervical Adeno-carcinoma (HeLa)	Cerikan, B. & Schiebel, E. [55]
Rab-GDI2	ISGylation of GDI2 alters Rab localization and promotes EGFR recycling, resulting in PI3K/Akt signaling	Proliferation, motility	Breast cancer	Bolado-Carrancio, A. [54]
KRAS	ISGylation stabilizes KRAS by limiting lysosomal degradation	Cytoskeletal disruption with altered morphology and increased migratory behavior	Breast cancer	Burks, J. [57]

3.2.5. Complex Regulation of the ISG15-PD-L1 Axis

Immune checkpoint blockade targeting PD-L1 has improved outcomes in several cancers; however, only a subset of patients respond, in part because of diverse intrinsic and acquired mechanisms of therapeutic resistance [58]. Therefore, understanding how PD-L1 expression and localization are regulated is critical. ISG15 has emerged as a potential regulator of this process.

Although most studies focus on surface PD-L1, which suppresses T-cell activity through PD-1 binding, PD-L1 localization is dynamic. Tumor cells may internalize PD-L1 or retain it at the plasma membrane, but the mechanisms controlling this trafficking remain incompletely understood. Because membrane trafficking depends on actin remodeling, vesicle transport, and endosomal recycling, cytoskeletal pathways regulated by ISG15 may influence PD-L1 localization.

ISG15 and ISGylation also play a role in receptor trafficking and oncogenic signaling. In basal breast cancer, ISGylation enhances EGFR recycling, prevents receptor degradation, and sustains Akt/KRAS signaling, thereby promoting tumor progression [54]. Because EGFR and PI3K-Akt-mTOR signaling can promote PD-L1 expression and immune evasion in cancers such as NSCLC [59,60], ISG15-mediated trafficking may indirectly regulate PD-L1 expression or localization.

Evidence across cancer types supports a context-dependent relationship between ISG15 and PD-L1. In gastric cancer, TAGLN2 activates the cGAS-STING/YBX1 pathway, inducing interferon-stimulated genes, including ISG15, and increasing PD-L1 expression. This axis correlates with poor survival and therapy resistance, suggesting that ISG15-associated interferon programs may contribute to PD-L1-mediated immune evasion [41]. Similarly, in pancreatic ductal adenocarcinoma, ISG15 is upregulated and promotes PD-L1 expression. ISG15 knockdown reduces PD-L1 expression, increases CD8+ T-cell infiltration, decreases regulatory T cells, suppresses tumor growth, and improves response to anti-PD-1 therapy [61].

However, ISG15 can also exert anti-tumor effects. In lung adenocarcinoma, ISG15 promotes degradation of glycosylated PD-L1 through the ubiquitin-proteasome pathway, reducing PD-L1 levels and enhancing T-cell infiltration. In mouse models, ISG15 overexpression combined with anti-PD-L1 therapy improves survival [62]. Similarly, in ovarian cancer, high coexpression of ISG15 and PD-L1 is associated with increased tumor mutation burden, greater CD8+ T-cell and M1 macrophage infiltration, and improved survival, suggesting a potential predictive marker for immunotherapy response [63].

Pan-cancer analyses further show that ISG15 is frequently overexpressed and associated with poor prognosis in colon, gastric, and pancreatic cancers. At the same time, ISG15 expression correlates with higher PD-L1 levels and improved responses to PD-1/PD-L1 blockade in some cohorts, including urothelial and lung cancers [64]. Thus, ISG15 has paradoxical and context-dependent roles in tumor progression and immunotherapy response.

Our group similarly reported that in EAC, high ISG15 expression correlates with poor prognosis, which is mediated via GRAIL-mediated CD3 degradation [38]; however, the relationship between ISG15 and PD-L1 expression in EAC remains unknown and is currently under investigation in our lab. Overall, ISG15 may regulate PD-L1 through interferon signaling, receptor trafficking, Akt/mTOR pathways, and cytoskeletal remodeling. Whether ISG15-driven cytoskeletal changes directly control PD-L1 trafficking and surface retention remains an important unanswered question. Figure 2 and Table 2 summarize such diverse findings that warrant further investigation.

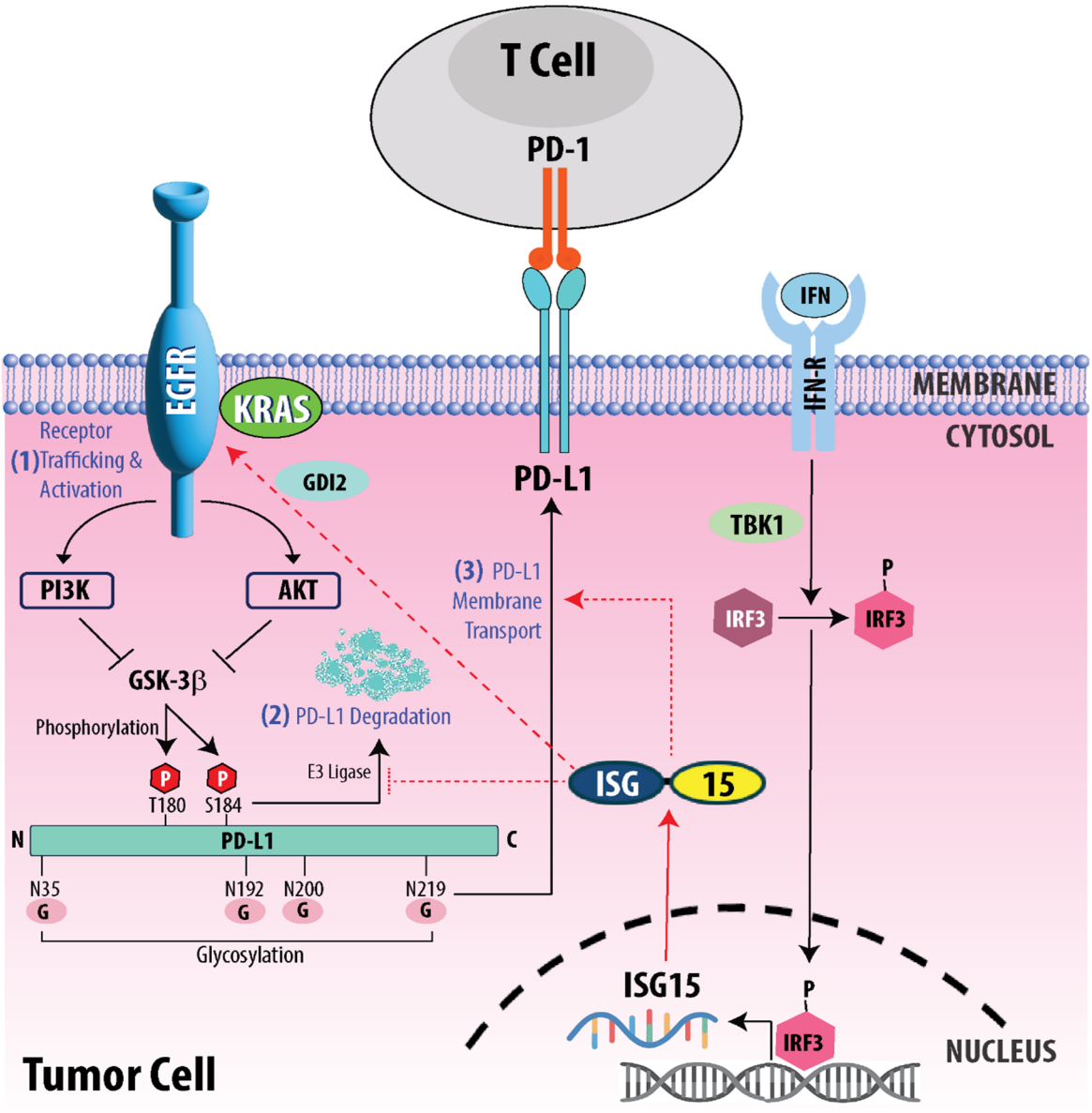

Figure 2. Context-dependent role of ISG15 in regulating PD-L1 expression in tumor cells. Chronic inflammation can promote tumor progression through altered interferon (IFN) signaling and activation of the TBK1-IRF3 pathway. Phosphorylated IRF3 translocates to the nucleus and induces interferon-stimulated genes (ISGs), including ISG15. Elevated ISG15 expression has been associated with poor prognosis and immune evasion in some tumor contexts. The figure presents a proposed mechanism linking ISG15 and ISGylation to PD-L1 regulation; dotted lines indicate hypothetical connections that require future validation. (**1**) ISGylation of GDI2 may favor EGFR recycling over lysosomal degradation, sustaining EGFR-PI3K-AKT signaling and promoting tumor-cell survival and proliferation. (**2**) Activation of this signaling axis may inhibit GSK-3β, thereby reducing PD-L1 phosphorylation at threonine 180 and serine 184, modifications that normally promote E3 ubiquitin ligase-dependent degradation. (**3**) N-glycosylation also contributes to PD-L1 stability, membrane trafficking, and surface expression. Together, these processes may influence PD-L1-mediated immune evasion, although their dependence on ISG15 remains to be established in the relevant tumor models.

Table 2. ISG15 and PD-L1 in Cancer.

ISG15-Associated Pathways	ISG15-Associated Mechanism	Expected Outcome	Classification	Cancer	Source
EGFR/Akt-mTOR/PD-L1 axis	ISG15-mediated receptor trafficking enhances Akt-mTOR signaling and alters PD-L1 membrane localization	Potential increase in PD-L1 expression and immune evasion	Indirect association/proposed mechanism	Breast cancer	Bolado-Carrancio, A. [54], Chen, N. [59], Lastwika, K.J. [60]
Interferon signaling	ISG15 is co-upregulated with IRDS and PD-L1 due to TAGLN2 interferon signaling	Immune evasion with therapy resistance and poorer survival	Indirect association	Gastric cancer	Zhuo, H. [41]
PD-L1 Expression Regulation	ISG15 promotes PD-L1 expression and immune suppression	Increased T-cell infiltration and reduced tumor growth following ISG15 knockdown	Experimental evidence	Pancreatic ductal adeno-carcinoma (PDAC)	Burks, J. [61]
Post-translational PD-L1 regulation	ISG15 promotes degradation and reduces stability of glycosylated PD-L1	Increased T-cell infiltration and reduced tumor growth	Experimental evidence	Lung adeno-carcinoma (LUAD)	Qu, T. [62]
Immune system	High ISG15 correlates with up-regulated PD-L1 expression and activation of the immune system	Immunologically active microenvironment with potential responsiveness to immunotherapy	Indirect association	Ovarian cancer	Luo, W. [63]
Immune checkpoint	ISG15 expression positively correlates with PD-L1 across many cancers	Poorer baseline prognosis but improved checkpoint inhibitor response	Indirect association	Pan-cancer analyses	Wei, J. [64]

4. Translational Value of Targeting the ISG15–Cytoskeletal Axis

4.1. Cytoskeletal Proteins as Therapeutic Targets in Cancers

Cytoskeletal remodeling is a fundamental requirement for tumor cell proliferation, invasion, metastasis, and survival under therapeutic stress. Alterations in microfilaments, microtubules, and intermediate filaments not only drive disease progression but also create distinct vulnerabilities that may be therapeutically exploited. Thus, targeting cytoskeletal mechanisms represents a rational strategy to suppress tumor growth, limit metastatic dissemination, and overcome therapy resistance.

4.1.1. Microfilaments: Targeting Actin Dynamics and Invasion

Actin microfilaments are central regulators of cancer cell motility, invasion, and EMT. Because actin itself is essential for normal cellular function, therapeutic strategies aimed at the actin cytoskeleton generally focus on actin-regulatory proteins rather than directly targeting actin filaments. In tumors, dysregulation of actin-binding and actin-regulatory proteins, including fascin, transgelins, and tropomyosins, supports invasive behavior and is often associated with poor prognosis [3].

In colorectal cancer, overexpression of tropomyosin-4, or TPM4, remodels actin stress fibers and enhances invasion, in part through loss of miR-133a-mediated repression [65]. These findings highlight actin–tropomyosin interactions as a mechanistically defined vulnerability in various cancers. Similarly, actin-bundling proteins such as fascin-1 promote filopodia and invadopodia formation and are associated with aggressive tumor phenotypes [28]. Although direct pharmacologic inhibition of actin regulators in cancer remains

largely preclinical, these studies establish actin remodeling as a promising therapeutic axis for suppressing invasion and metastasis.

4.1.2. Microtubules: Established and Emerging Therapeutic Targets

Microtubules are the most clinically validated cytoskeletal targets in cancer therapy. They play essential roles in mitosis, intracellular trafficking, and maintenance of cell polarity. Microtubule-targeting agents (MTAs) remain important components of systemic cancer therapy, particularly in gastric and colorectal malignancies.

Classical MTAs, including taxanes and vinca alkaloids, disrupt microtubule dynamics, leading to mitotic arrest and apoptosis [66–71]. However, resistance frequently emerges through mechanisms such as altered tubulin isotype expression and changes in tubulin PTMs. Newer agents, such as eribulin, suppress microtubule growth at plus ends and influence not only mitosis but also EMT-related phenotypes and tumor microenvironment remodeling [72–75]. These properties suggest that MTAs may function not only as cytotoxic agents but also as anti-metastatic or "migrastatic" therapies in cancer.

4.1.3. Intermediate Filaments: Targeting Vimentin and EMT-Associated Plasticity

Intermediate filaments, particularly vimentin, play critical roles in EMT, mechanical resilience, and therapy resistance in cancer. Vimentin expression is strongly associated with mesenchymal phenotypes, enhanced migration, and poor clinical outcomes. Its upregulation stabilizes the morphology of invasive cells and supports cytoskeletal crosstalk with actin filaments and microtubules.

Targeting vimentin has emerged as a promising strategy to suppress EMT-driven invasion and potentially resensitize tumors to therapy. Pharmacologic disruption of vimentin assembly or function reduces motility and partially reverses mesenchymal traits in many cancer models [76]. Importantly, vimentin-associated cytoskeletal states have also been linked to resistance to radiation and chemotherapy, suggesting that intermediate filament-directed approaches may act as radiosensitizers or chemosensitizers in EMT-high tumors [77].

4.1.4. Cytoskeletal Crosstalk as an Emerging Therapeutic Avenue

Although individual cytoskeletal systems represent valid therapeutic targets, increasing evidence suggests that crosstalk among microfilaments, microtubules, and intermediate filaments is equally important for cancer progression. Proteins such as formins, vimentin-associated regulators, and microtubule-modifying enzymes coordinate the integration of these systems, enabling adaptive responses to therapeutic stress [28,78]. Targeting these integrative nodes may provide a way to simultaneously disrupt multiple cytoskeletal functions, thereby limiting compensatory mechanisms and reducing resistance.

Overall, cytoskeletal proteins constitute a mechanistically grounded and clinically relevant class of therapeutic targets. Actin-regulatory pathways drive invasion and metastasis, microtubules remain central targets of systemic chemotherapy, and intermediate filaments such as vimentin support EMT and therapy resistance. Emerging strategies that target cytoskeletal crosstalk hold particular promise for overcoming resistance and improving therapeutic durability. Table 3 summarizes prominent examples where cytoskeletal proteins have been targeted therapeutically.

Table 3. Cytoskeletal Proteins as Therapeutic Targets.

Therapy/Therapeutic Strategy	Primary Cytoskeletal Target	Mechanism/Location of Action	Sources
Transgelin (TAGLN/TAGLN2) modulation	Actin microfilaments	Alter actin stability and cytoskeletal plasticity linked to EMT and motility in cancers.	Dvorakova M. [29], Kim, H. [79], Thompson, O. [80]
TPM4-associated targeting strategies	Actin microfilaments	Modulate actin stress fiber formation and contractility; TPM4 overexpression enhances invasion in colorectal cancer	Caporali, S. [65]
Fascin-directed strategies	Actin microfilaments	Inhibit actin bundling in filopodia and invadopodia, reducing migration and ECM invasion.	Sarantelli, E. [28]
Taxanes	Microtubules	Stabilize β-tubulin polymers, suppress microtubule dynamics, induce mitotic arrest, and apoptosis in proliferating tumor cells.	Skubnik, J. [67]
Vinca Alkaloids	Microtubules	Destabilize microtubules, disrupt spindle formation, inhibit cell division, and tumor growth.	Wang, R. [68], Bates, D. [69] Moudi, M. [70], Banyal, A. [71]
Eribulin	Microtubules	Inhibit microtubule plus-end growth, alter mitosis and EMT-associated phenotypes, and reduce invasive behavior.	Chanez, B. [72], Helfrich, B. [73], Hüsemann, L. [74], Eslamian, G. [75]
Vimentin-targeting agents	Intermediate Filaments	Disrupt vimentin assembly and mesenchymal cytoskeletal architecture; suppress EMT-driven migration and resistance; reduce mechanical resilience, invasiveness, and therapy resistance associated with mesenchymal states	Strouhalova, K. [76], Čermák, V. [77]
ALD-R491 (small molecule)	Intermediate Filaments	Bind to vimentin, stabilizing filaments, reducing cell migration, and reversing EMT phenotypes	Rosier, M. [81]
Withaferin-A (WFA)	Intermediate Filaments	Bind to vimentin, induce apoptosis, and exhibit significant anti-angiogenic effects on cancer cells	Bargagna-Mohan, P. [82]

4.2. Potential ISG15-Associated Cytoskeletal Targets for Therapeutic Intervention

Although growing evidence links ISG15 to cancer progression and responses to standard therapies, the therapeutic potential of the ISG15–cytoskeleton relationship remains underexplored. Current cytoskeleton-targeting therapies can be effective, but they are often limited by systemic toxicity and acquired resistance. In contrast, ISG15 may regulate more selective cytoskeletal substrates and signaling networks preferentially activated in aggressive tumor states. Therefore, focusing on the ISG15–cytoskeleton axis may reveal more precise therapeutic opportunities. Central to this effort is identifying relevant ISG15-interacting substrates and determining how these interactions influence tumor behavior, metastatic potential, and resistance phenotypes.

One compelling ISG15-associated target is NMIIA. ISG15 directly interacts with NMIIA in the context of breast cancer plasticity, and NMIIA also functions as an oncogenic regulator in pancreatic, gastric, colorectal, and esophageal cancers, where it contributes to chemoradiotherapy resistance [83]. Because NMIIA has resistance-promoting roles across multiple cancer types and its interaction with ISG15 has already been characterized, the ISG15-NMIIA axis represents one of the most relevant cytoskeletal pathways for future investigation.

Another potential protein of interest is HDAC6, a cytoplasmic deacetylase that influences cytoskeletal remodeling and cancer progression [10,84,85]. HDAC6 associates with microtubules and the microtubule motor complex to regulate microtubule-dependent cell movement [86]. It also deacetylates cortactin, an F-actin-binding protein involved in actin-dependent motility. Cortactin is upregulated in several cancers and enriched in invasive breast cancer cells, where it facilitates extracellular matrix degradation [84]. HDAC6 also modulates tumor cell migration and self-renewal through Rac1-dependent cytoskeletal dynamics [87] and has been associated with oncogenic transformation [88]. Given these functions, the interaction between ISG15 and HDAC6 may represent a mechanism through which ISG15 influences cytoskeletal architecture and therapeutic resistance, although this relationship remains to be fully characterized.

Here, we highlight some proteins identified through mass-spectrometry-based proteomic screens that have potential sites for ISG15 modification [89]. We also briefly summarize what is known about their role in cancer progression and resistance, making them attractive targets for future studies. For example, components of the Arp2/3 complex, including ARPC5, ACTR2, and ACTR3, may interact with ISG15 to regulate actin polymerization, a process critical for EMT and invasion [89]. Arp2/3 activity is required for tumor microtube formation in pancreatic cancer, a mechanism associated with therapy resistance [90]. Therefore, whether ISG15 regulates Arp2/3 complex activity remains an open and highly relevant question in cancer biology.

Moesin, also known as membrane-organizing extension spike protein, represents another potential cytoskeletal target associated with ISG15. As a member of the ERM protein family, moesin links the plasma membrane to the actin cytoskeleton [91]. Moesin has established roles in tumor survival and metastasis [92] and is enriched in aggressive breast cancer subtypes, including estrogen receptor-negative and triple-negative tumors [93]. Its expression has also been linked to drug resistance, as moesin silencing restores breast cancer sensitivity to doxorubicin [94]. Whether the interaction between ISG15 and moesin influences resistant phenotypes is unknown.

Another compelling protein for future study is α-tubulin, which has been reported to undergo ISGylation [95]. α-tubulin is extensively regulated by PTMs, including acetylation, and has been linked to cancer progression, chemosensitivity, and metastasis [35,96]. Since ISGylation sites have some potential overlap with established acetylation sites [89], there may be interactions between ISGylation and acetylation in regulating microtubule stability and drug response.

A broader class of potential targets includes actin-binding proteins such as filamin-A, or FLNA, and α-actinin-4, or ACTN4. Both are potential ISG15 interaction partners, but their functional relationships with ISG15 in resistance contexts remain uncharacterized [89]. FLNA enhances DNA repair capacity and is associated with increased drug resistance in melanoma models [97], while ACTN4 promotes colorectal cancer motility and metastasis [98]. Future studies examining ISG15 interactions with FLNA and ACTN4 may help define the broader influence of ISG15 on actin-dependent resistance mechanisms.

Although proteomic approaches can identify candidate ISG15-associated proteins, functional studies in cancer models are needed to understand how ISG15 interacts with these cytoskeletal proteins and how these interactions contribute to therapeutic resistance. Future studies should confirm these ISG15 interactions and explore their functional consequences through biochemical and mechanistic validation. Additionally, it is important to emphasize that free ISG15 can interact with other biomolecules independently of covalent ISGylation; therefore, proteomic approaches focused on identifying potential ISGylation sites may not capture the full repertoire of biomolecules whose functions are influenced by ISG15. Future research should characterize both covalent and non-covalent ISG15 interactions

and investigate their functional consequences in treatment-resistant cancer models. These studies will advance our understanding of whether and how ISG15-mediated interactions with cytoskeletal proteins influence cell survival and treatment response.

4.3. Current ISG15-Targeting Therapeutic Strategies

To date, no ISG15-targeting therapy has been approved or widely implemented for cancer treatment. Direct pharmacological inhibition of ISG15 itself remains highly challenging. Unlike conventional drug targets such as kinases, proteases, or other enzymes, ISG15 is a small ubiquitin-like protein modifier that lacks intrinsic catalytic activity and therefore does not contain a classical enzymatic active site that can be readily targeted by small molecules. Structurally, ISG15 consists of two ubiquitin-like β-grasp domains connected by a flexible linker and shares substantial structural similarity with ubiquitin, including the conserved β-grasp fold and C-terminal LRLRGG motif. This structural similarity may further complicate the development of selective ISG15-targeting agents while minimizing potential cross-reactivity with the ubiquitin system. In addition, ISG15 lacks well-defined small-molecule binding pockets and presents a relatively flat molecular surface. Its biological functions are mediated predominantly through covalent conjugation to numerous substrate proteins and through noncovalent protein–protein interactions involving relatively large and shallow interaction surfaces, which are inherently difficult to disrupt using conventional small molecules. Consequently, direct inhibition of ISG15 would likely require selective disruption of specific protein–protein interaction interfaces or interference with its C-terminal recognition and conjugation machinery rather than occupation of a conventional catalytic pocket. Further complicating direct targeting, ISG15 exists in several biologically distinct forms, including free intracellular ISG15, secreted extracellular ISG15, and protein-conjugated ISG15, which can exert context- and tumor-dependent pro-tumor or anti-tumor effects. Thus, global inhibition of ISG15 could simultaneously suppress oncogenic ISGylation while disrupting beneficial antiviral or anti-tumor immune functions. Collectively, these structural and biological challenges have shifted therapeutic development toward more pharmacologically tractable regulators of ISGylation, including the host enzymes UBA7, UBE2L6, HERC5, and USP18, as well as viral deISGylating enzymes such as coronavirus papain-like protease (PLpro) and disease-specific ISG15–substrate interactions.

Therapeutic strategies that modulate ISG15-related pathways have been explored extensively in viral infections, particularly SARS-CoV-2. These studies provide a useful framework for considering how ISG15-directed approaches might eventually be adapted for oncology. One major viral target connected to ISG15 regulation is the coronavirus PLpro, a multifunctional viral protease that contributes to both viral polyprotein processing and immune evasion. In addition to its essential role in viral replication, PLpro counteracts ISG15-mediated antiviral defense through its deISGylating activity. Specifically, PLpro enzymatically cleaves ISG15 from conjugated host proteins, thereby reversing protein ISGylation. This disrupts an important component of the antiviral response, as ISGylation modifies numerous cellular and viral proteins involved in innate immune signaling and restriction of viral replication. PLpro removes ISG15 from key immune regulators, including IRF3 and MDA5, thereby attenuating interferon signaling and antiviral sensor activation. In addition, PLpro-mediated deISGylation also increases the levels of free, unconjugated ISG15, which may further dysregulate immune responses by altering cytokine signaling dynamics. In particular, increased extracellular free ISG15 has been associated with macrophage activation and enhanced secretion of pro-inflammatory cytokines and chemokines, including IL-6, TNF-α, IL-1β, and MCP-1, while concomitantly reducing type I and type II interferon secretion during SARS-CoV-2 infection. Altogether, PLpro-mediated disruption of the balance between protein-conjugated and free ISG15 contributes to both suppression of

antiviral interferon responses and dysregulated inflammatory signaling, facilitating viral immune evasion and pathogenesis [99].

Although deISGylating activity is conserved among several coronavirus PLpro enzymes, their relative substrate preferences for ISG15 and ubiquitin can differ substantially. Structural studies have shown that PLpro recognizes ISG15 through defined ubiquitin-like protein-binding interfaces, enabling efficient binding and hydrolysis of ISG15-conjugated substrates [99]. This substrate recognition provides a structural basis for PLpro-mediated disruption of ISG15-dependent antiviral defenses. Mechanistically, PLpro-mediated deISGylation disrupts innate immune signaling by removing ISG15 from key regulators such as IRF3 and MDA5, thereby suppressing interferon-mediated antiviral responses [100]. PLpro can also target viral proteins, such as nucleocapsid, where ISGylation would otherwise inhibit viral assembly or replication. Thus, removal of ISG15 from these substrates directly promotes viral fitness [101]. Notably, some coronavirus PLpro enzymes preferentially bind and cleave ISG15 over ubiquitin substrates, underscoring ISGylation as a key antiviral pathway targeted by viral immune evasion strategies [102].

These findings have motivated the development of PLpro inhibitors designed to block viral deISGylation, preserve protein ISGylation, and restore antiviral immune responses. One promising PLpro inhibitor is GRL0617, which has shown antiviral activity in cell-based assays by occupying the BL2 substrate-binding groove and interfering with recognition and processing of ISG15 and other PLpro substrates [103,104]. Although these agents have primarily been studied in viral infection models, they demonstrate that pharmacologic modulation of ISG15-related enzymatic pathways is feasible. Recently, our group reported the synthesis of potent, selective covalent inhibitors of PLpro, viz., GL-508, GL-612, GL-637 and GL-642, designed to exploit Glu167 within the ISG15/ubiquitin-recognition region together with the BL2 hydrophobic groove, thereby disrupting substrate recognition and PLpro function. These compounds showed potent enzymatic inhibition and antiviral activity with minimal cytotoxicity [105]. Importantly, although these compounds are PLpro inhibitors rather than direct ISG15 or ISGylation inhibitors, their ability to pharmacologically target an ISG15-recognition interface provides a valuable structural framework for the future development of agents that selectively modulate ISG15-dependent pathways. Such strategies could potentially be explored in oncology as therapy-sensitizing approaches in tumors driven by aberrant ISG15/ISGylation signaling.

A second therapeutic strategy involves targeting USP18, a host deISGylating enzyme that removes ISG15 from conjugated proteins. In contrast to strategies that block ISGylation, USP18 inhibition increases ISGylation by preventing ISG15 removal from target proteins. Tanshinone IIA sulfonate, or TST, has been identified as a USP18 inhibitor that can enhance ISGylation, destabilize cancer-promoting proteins, and trigger apoptosis. TST has also been reported to increase STING protein levels, thereby enhancing anti-tumor immune responses, particularly when combined with STING agonists such as diABZi [106]. More recently, high-throughput screening identified β-lapachone and related quinone compounds as small-molecule inhibitors of USP18 enzymatic activity. β-Lapachone increased global ISGylation, reduced lung cancer cell proliferation, promoted apoptosis, and suppressed tumor growth in a lung cancer xenograft model. Structural modeling and mutational analyses further identified a hydrophobic USP18 substrate-recognition pocket involving D139, F259, and T266 that may provide a tractable site for future structure-based inhibitor development [107]. These findings suggest that USP18 inhibition may provide a potential route for increasing ISGylation-dependent anti-tumor activity, though this depends on the context of the cancer.

Overall, although ISG15-targeting approaches are in the pre-clinical development stage and have not yet been established in cancer therapy, studies in viral infection and emerg-

ing cancer models suggest that ISG15-related enzymes are pharmacologically tractable. Translating these strategies to oncology may provide a meaningful approach to modulating ISG15-dependent protein modifications, particularly in tumors where ISG15 promotes metastatic phenotypes, immune evasion, or therapeutic resistance.

4.4. Translational Implications and Challenges

Given its relevance in cancer progression, immune regulation, and treatment response, ISG15 is an important candidate for translational investigation. ISG15 may have clinical utility as a therapeutic target or as a biomarker to predict treatment response and guide patient stratification.

Preclinical studies also support the potential of ISG15-directed immunotherapy. In mouse models, vaccination with an ISG15-targeting *Listeria monocytogenes*-based cytotoxic T lymphocyte vaccine, Lm-LLO-ISG15, induced CD8+ T-cell responses that reduced breast tumor growth and metastasis [108]. Similarly, in renal cell carcinoma, ISG15 has been shown to function as a tumor-associated antigen. Lm-LLO-ISG15 vaccination increased CD4+ and CD8+ T-cell infiltration, and its therapeutic effects were comparable to anti-PD-1 therapy and sunitinib [109]. These findings highlight the potential of ISG15-directed immunotherapy, particularly in tumors where ISG15 is highly expressed and immunologically accessible.

The relationship between ISG15 and PD-L1 further raises the possibility that ISG15 may be linked to broader immune checkpoint regulation. Future studies should determine whether ISG15 is associated with other checkpoint molecules, such as TIM-3, or with responsiveness to emerging immunotherapies, including CAR-T cell therapy. In this context, ISG15 may have value as both a prognostic biomarker and a predictor of differential immunotherapy response. If validated, ISG15-based testing could help guide more personalized treatment strategies in oncology.

Despite this translational promise, several major challenges remain. First, ISG15 has context-dependent roles in cancer. Elevated ISG15 expression has been associated with both favorable and unfavorable outcomes depending on tumor type, treatment setting, and immune context. Therefore, ISG15 cannot be interpreted simply as either a pro-tumor or anti-tumor marker. More work is needed to define how ISG15 function varies according to cancer type, disease stage, molecular subtype, genetic background, and therapy exposure. ISG15 will also need to be evaluated alongside established biomarkers, including tumor mutational burden, PD-L1 expression, interferon-related gene signatures, and other clinically validated molecular markers.

Another challenge is the lack of standardization in how ISG15 is measured. Current studies assess ISG15 across different sample sources, including tumor tissue, plasma, and cell lysates, each of which captures distinct biological information. In addition, ISG15 can be measured as free ISG15, conjugated ISG15, total ISG15 protein, or ISG15 mRNA. These different readouts may reflect different biological states and are not necessarily interchangeable. This variability makes it difficult to compare findings across studies and complicates efforts to establish a clear and reproducible interpretation of ISG15's role in cancer.

5. Conclusions, Outlook and Future Directions

The evidence reviewed here supports a complex model in which ISG15 may contribute to therapy resistance through regulation of cytoskeletal architecture, cellular plasticity, DNA damage repair, receptor trafficking, oncogenic signaling, and immune checkpoint pathways. However, ISG15 biology is highly context-dependent. In some tumor types, ISG15 appears to promote resistance, metastasis, and immune evasion, whereas in others it may enhance chemosensitivity or anti-tumor immunity.

A major gap in the field is the limited functional characterization of ISG15-associated cytoskeletal substrates in aggressive cancers. Future studies should systematically define the ISG15 interactome and ISGylome across therapy-sensitive and therapy-resistant tumors. Further studies are needed to understand how ISG15 interacts with cytoskeletal proteins and how these interactions alter cell morphology, migration, invasion, DNA repair, immune evasion, and treatment response. It is also important to distinguish the different fates of ISG15, such as the covalently conjugated (ISGylated) ISG15 or the secreted ISG15, and determine how these different fates may contribute to therapeutic resistance.

Several key questions remain unresolved. First, does ISG15-mediated cytoskeletal remodeling directly cause therapeutic resistance, or does it primarily mark resistant cellular states? Second, do free ISG15 and conjugated ISG15 have distinct or opposing effects on tumor progression? Third, can ISG15-associated cytoskeletal signatures predict response to chemotherapy, radiotherapy, or immune checkpoint blockade? Finally, can pharmacologic targeting of ISG15-related enzymes, such as PLpro, USP18, or other regulators of ISGylation, be used safely and effectively in cancer?

Future research identifying proteins and molecules that interact with ISG15 will be essential for understanding how these interactions contribute to cytoskeletal remodeling, cancer progression, and therapeutic resistance. Further research is also needed to determine which cancer types and treatment settings are most appropriate for ISG15 testing, which molecular form of ISG15 provides the most clinically meaningful information, and whether ISG15 offers predictive or prognostic value beyond existing biomarkers. Although existing studies have reported associations between ISG15 and proteins involved in these processes, establishing causality and clarifying the underlying mechanisms are important unresolved challenges in the field. Addressing these questions will be critical for determining whether ISG15 can be translated into a clinically useful therapeutic target or biomarker in oncology. The ISG15–cytoskeletal axis may offer new opportunities to overcome therapeutic resistance.

Study Selection

Studies in the literature were identified through searches of PubMed, Google Scholar, and ScienceDirect using keywords related to ISG15, cancer, cytoskeletal regulation, therapeutic resistance, and treatment response. Studies were selected based on their relevance to the topics discussed in this review. Wherever necessary, we analyzed supplementary data from the original article to identify potential post-translational modification sites. Review articles were used to identify additional relevant studies and provide broader context. As this is a narrative review, study selection may not be exhaustive or systematic.

Author Contributions: Conceptualization, S.P., N.V., N.S.G. and D.R.; methodology, S.P. N.V., N.S.G. and D.R.; investigation, S.P., N.V. N.S.G. and D.R.; resources, N.S.G. and D.R.; writing-original draft preparation, S.P., N.V., N.S.G. and D.R.; writing-review and editing, S.P., N.V. and D.R.; visualization, S.P., N.V., N.S.G. and D.R.; supervision, D.R.; project administration, D.R.; funding acquisition, N.S.G. and D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by R01 CA215596, P50 CA269022, and G002823 (Thomas Charles M. Esophageal Cancer Endowed Research Fund to D. Ray). This work is partially supported by the National Institutes of Health, United States (R01 CA247370 and R01 AI161570), to N. Gavande.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We thank members of the Ray and Gavande laboratories for critical insights and discussion during the preparation of this article. We regret our inability to cite many relevant publications that helped in our initial conceptualization and idea development. The authors also acknowledge the use of University of Michigan GPT-6.1 Sol and Claude AI (model Sonet 5.5) for

assistance with manuscript editing to improve clarity. The authors acknowledge limited use of Biorender.com free accounts by S.P., N.V. and D.R. to generate models in figures.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cheng, X.; Xu, M.; Wei, J.; Cao, C. Cancer Drug Response and Resistance: Molecular Mechanisms and Combating Strategies. *Signal Transduct. Target. Ther.* **2026**, *11*, 307. [CrossRef] [PubMed]
2. Momotyuk, E.; Ebrahim, N.; Shakirova, K.; Dashinimaev, E. Role of the Cytoskeleton in Cellular Reprogramming: Effects of Biophysical and Biochemical Factors. *Front. Mol. Biosci.* **2025**, *12*, 1538806. [CrossRef] [PubMed]
3. Lim, S.; Woo, S.; Lee, K.W.; Kim, K.D. Roles of Cytoskeleton in Metastasis: From Its Mechanism to Therapeutic Strategies. *Exp. Mol. Med.* **2026**, *58*, 1–13. [CrossRef] [PubMed]
4. Aseervatham, J. Cytoskeletal Remodeling in Cancer. *Biology* **2020**, *9*, 385. [CrossRef] [PubMed]
5. Ong, M.S.; Deng, S.; Halim, C.E.; Cai, W.; Tan, T.Z.; Huang, R.Y.-J.; Sethi, G.; Hooi, S.C.; Kumar, A.P.; Yap, C.T. Cytoskeletal Proteins in Cancer and Intracellular Stress: A Therapeutic Perspective. *Cancers* **2020**, *12*, 238. [CrossRef] [PubMed]
6. Datta, A.; Deng, S.; Gopal, V.; Yap, K.C.-H.; Halim, C.E.; Lye, M.L.; Ong, M.S.; Tan, T.Z.; Sethi, G.; Hooi, S.C.; et al. Cytoskeletal Dynamics in Epithelial-Mesenchymal Transition: Insights into Therapeutic Targets for Cancer Metastasis. *Cancers* **2021**, *13*, 1882. [CrossRef] [PubMed]
7. De Las Rivas, J.; Brozovic, A.; Izraely, S.; Casas-Pais, A.; Witz, I.P.; Figueroa, A. Cancer Drug Resistance Induced by EMT: Novel Therapeutic Strategies. *Arch. Toxicol.* **2021**, *95*, 2279–2297. [CrossRef] [PubMed]
8. Winkler, J.; Abisoye-Ogunniyan, A.; Metcalf, K.J.; Werb, Z. Concepts of Extracellular Matrix Remodelling in Tumour Progression and Metastasis. *Nat. Commun.* **2020**, *11*, 5120. [CrossRef] [PubMed]
9. Kang, J.A.; Kim, Y.J.; Jeon, Y.J. The Diverse Repertoire of ISG15: More Intricate than Initially Thought. *Exp. Mol. Med.* **2022**, *54*, 1779–1792. [CrossRef] [PubMed]
10. Perng, Y.-C.; Lenschow, D.J. ISG15 in Antiviral Immunity and Beyond. *Nat. Rev. Microbiol.* **2018**, *16*, 423–439. [CrossRef] [PubMed]
11. Nguyen, H.-M.; Gaikwad, S.; Oladejo, M.; Agrawal, M.Y.; Srivastava, S.K.; Wood, L.M. Interferon Stimulated Gene 15 (ISG15) in Cancer: An Update. *Cancer Lett.* **2023**, *556*, 216080. [CrossRef] [PubMed]
12. Bogunovic, D.; Boisson-Dupuis, S.; Casanova, J.-L. ISG15: Leading a Double Life as a Secreted Molecule. *Exp. Mol. Med.* **2013**, *45*, e18. [CrossRef] [PubMed]
13. Desai, S.D. ISG15: A Double Edged Sword in Cancer. *Oncoimmunology* **2015**, *4*, e1052935. [CrossRef] [PubMed]
14. Wardlaw, C.P.; Petrini, J.H.J. ISG15: A Link between Innate Immune Signaling, DNA Replication, and Genome Stability. *BioEssays* **2023**, *45*, 2300042. [CrossRef] [PubMed]
15. Sandy, Z.; Da Costa, I.C.; Schmidt, C.K. More than Meets the ISG15: Emerging Roles in the DNA Damage Response and Beyond. *Biomolecules* **2020**, *10*, 1557. [CrossRef] [PubMed]
16. Hohmann, T.; Dehghani, F. The Cytoskeleton—A Complex Interacting Meshwork. *Cells* **2019**, *8*, 362. [CrossRef] [PubMed]
17. Winder, S.J.; Ayscough, K.R. Actin-Binding Proteins. *J. Cell Sci.* **2005**, *118*, 651–654. [CrossRef] [PubMed]
18. McGough, A. F-Actin-Binding Proteins. *Curr. Opin. Struct. Biol.* **1998**, *8*, 166–176. [CrossRef] [PubMed]
19. Hartwig, J.H.; Kwiatkowski, D.J. Actin-Binding Proteins. *Curr. Opin. Cell Biol.* **1991**, *3*, 87–97. [CrossRef] [PubMed]
20. Goodson, H.V.; Jonasson, E.M. Microtubules and Microtubule-Associated Proteins. *Cold Spring Harb. Perspect. Biol.* **2018**, *10*, a022608. [CrossRef] [PubMed]
21. Lowery, J.; Kuczmarski, E.R.; Herrmann, H.; Goldman, R.D. Intermediate Filaments Play a Pivotal Role in Regulating Cell Architecture and Function. *J. Biol. Chem.* **2015**, *290*, 17145–17153. [CrossRef] [PubMed]
22. Kuburich, N.A.; Den Hollander, P.; Pietz, J.T.; Mani, S.A. Vimentin and Cytokeratin: Good Alone, Bad Together. *Semin. Cancer Biol.* **2022**, *86*, 816–826. [CrossRef] [PubMed]
23. Biber, G.; Ben-Shmuel, A.; Sabag, B.; Barda-Saad, M. Chapter Three—Actin Regulators in Cancer Progression and Metastases: From Structure and Function to Cytoskeletal Dynamics. In *Actin Cytoskeleton in Cancer Progression and Metastasis—Part B*; Thomas, C., Galluzzi, L., Eds.; International Review of Cell and Molecular Biology; Academic Press: Cambridge, MA, USA, 2020; Volume 356, pp. 131–196.
24. Friedman, E.; Verderame, M.; Lipkin, M.; Pollack, R. Altered Actin Cytoskeletal Patterns in Two Premalignant Stages in Human Colon Carcinoma Development. *Cancer Res.* **1985**, *45*, 3236–3242. [PubMed]
25. Friedman, E.; Verderame, M.; Winawer, S.; Pollack, R. Actin Cytoskeletal Organization Loss in the Benign-to-Malignant Tumor Transition in Cultured Human Colonic Epithelial Cells. *Cancer Res.* **1984**, *44*, 3040–3050. [PubMed]
26. Pratiwi, L.; Elisa, E.; Sutanto, H. Probing the Protrusions: Lamellipodia and Filopodia in Cancer Invasion and Beyond. *Mechanobiol. Med.* **2024**, *2*, 100064. [CrossRef] [PubMed]

27. Schaks, M.; Giannone, G.; Rottner, K. Actin Dynamics in Cell Migration. *Essays Biochem.* **2019**, *63*, 483–495. [CrossRef] [PubMed]
28. Sarantelli, E.; Mourkakis, A.; Zacharia, L.C.; Stylianou, A.; Gkretsi, V. Fascin-1 in Cancer Cell Metastasis: Old Target-New Insights. *Int. J. Mol. Sci.* **2023**, *24*, 11253. [CrossRef] [PubMed]
29. Dvorakova, M.; Nenutil, R.; Bouchal, P. Transgelins, Cytoskeletal Proteins Implicated in Different Aspects of Cancer Development. *Expert Rev. Proteom.* **2014**, *11*, 149–165. [CrossRef] [PubMed]
30. Aspenström, P.; Fransson, Å.; Saras, J. Rho GTPases Have Diverse Effects on the Organization of the Actin Filament System. *Biochem. J.* **2004**, *377*, 327–337. [CrossRef] [PubMed]
31. Nobes, C.D.; Hall, A. Rho, Rac, and Cdc42 GTPases Regulate the Assembly of Multimolecular Focal Complexes Associated with Actin Stress Fibers, Lamellipodia, and Filopodia. *Cell* **1995**, *81*, 53–62. [CrossRef] [PubMed]
32. Begum, R.; Nur-E-Kamal, M.S.A.; Zaman, M. The Role of Rho GTPases in the Regulation of the Rearrangement of Actin Cytoskeleton and Cell Movement. *Exp. Mol. Med.* **2004**, *36*, 358–366. [CrossRef] [PubMed]
33. Lopes, D.; Maiato, H. The Tubulin Code in Mitosis and Cancer. *Cells* **2020**, *9*, 2356. [CrossRef] [PubMed]
34. Rodrigues-Ferreira, S.; Morin, M.; Guichaoua, G.; Moindjie, H.; Haykal, M.M.; Collier, O.; Stoven, V.; Nahmias, C. A Network of 17 Microtubule-Related Genes Highlights Functional Deregulations in Breast Cancer. *Cancers* **2023**, *15*, 4870. [CrossRef] [PubMed]
35. Wattanathamsan, O.; Pongrakhananon, V. Post-Translational Modifications of Tubulin: Their Role in Cancers and the Regulation of Signaling Molecules. *Cancer Gene Ther.* **2023**, *30*, 521–528. [CrossRef] [PubMed]
36. Surolia, R.; Antony, V.B. Pathophysiological Role of Vimentin Intermediate Filaments in Lung Diseases. *Front. Cell Dev. Biol.* **2022**, *10*, 872759. [CrossRef] [PubMed]
37. Chauhan, R.; Gupta, A.; Dagar, G.; Sharma, S.; Sadida, H.Q.; Hashem, S.; Verghese, A.M.; Tanwar, M.; Macha, M.A.; Uddin, S.; et al. Role of Lamins in Cellular Physiology and Cancer. In *Advances in Protein Chemistry and Structural Biology*; Elsevier: Amsterdam, The Netherlands, 2025; Volume 143, pp. 119–153, ISBN 978-0-443-29564-5.
38. McEwen, D.P.; Ray, P.; Nancarrow, D.J.; Wang, Z.; Kasturirangan, S.; Abdullah, S.; Balan, A.; Hoskeri, R.; Thomas, D.; Lawrence, T.S.; et al. ISG15/GRAIL1/CD3 Axis Influences Survival of Patients with Esophageal Adenocarcinoma. *JCI Insight* **2024**, *9*, e179315. [CrossRef] [PubMed]
39. Ghosh, A.; Ray, P.; Thomas, D.; Jeeyar, V.; Pillai, S.; Basrur, V.; Bondarenko, I.V.; Bellile, E.; Wang, D.H.; Morgan, M.A.; et al. ISGylation of γH2AX Retains MDC1 and Facilitates Homologous Recombination Repair Causing Radioresistance in Esophageal Adenocarcinoma. *J. Biol. Chem.* **2026**, *302*, 111358. [CrossRef] [PubMed]
40. Ina, S.; Hirono, S.; Noda, T.; Yamaue, H. Identifying Molecular Markers for Chemosensitivity to Gemcitabine in Pancreatic Cancer: Increased Expression of Interferon-Stimulated Gene 15 Kd Is Associated With Intrinsic Chemoresistance. *Pancreas* **2010**, *39*, 473–485. [CrossRef] [PubMed]
41. Zhuo, H.; Hou, J.; Hong, Z.; Yu, S.; Peng, H.; Zhang, L.; Xie, W.; Hong, X. TAGLN2 Induces Resistance Signature ISGs by Activating AKT-YBX1 Signal with Dual Pathways and Mediates the IFN-Related DNA Damage Resistance in Gastric Cancer. *Cell Death Dis.* **2024**, *15*, 608. [CrossRef] [PubMed]
42. Shen, J.; Wei, J.; Wang, H.; Yue, G.; Yu, L.; Yang, Y.; Xie, L.; Zou, Z.; Qian, X.; Ding, Y.; et al. A Three-Gene Signature as Potential Predictive Biomarker for Irinotecan Sensitivity in Gastric Cancer. *J. Transl. Med.* **2013**, *11*, 73. [CrossRef] [PubMed]
43. Weichselbaum, R.R.; Ishwaran, H.; Yoon, T.; Nuyten, D.S.A.; Baker, S.W.; Khodarev, N.; Su, A.W.; Shaikh, A.Y.; Roach, P.; Kreike, B.; et al. An Interferon-Related Gene Signature for DNA Damage Resistance Is a Predictive Marker for Chemotherapy and Radiation for Breast Cancer. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18490–18495. [CrossRef] [PubMed]
44. Jeon, Y.J.; Jo, M.G.; Yoo, H.M.; Hong, S.-H.; Park, J.-M.; Ka, S.H.; Oh, K.H.; Seol, J.H.; Jung, Y.K.; Chung, C.H. Chemosensitivity Is Controlled by P63 Modification with Ubiquitin-like Protein ISG15. *J. Clin. Investig.* **2012**, *122*, 2622–2636. [CrossRef] [PubMed]
45. Desai, S.D.; Wood, L.M.; Tsai, Y.-C.; Hsieh, T.-S.; Marks, J.R.; Scott, G.L.; Giovanella, B.C.; Liu, L.F. ISG15 as a Novel Tumor Biomarker for Drug Sensitivity. *Mol. Cancer Ther.* **2008**, *7*, 1430–1439. [CrossRef] [PubMed]
46. Tessema, M.; Yingling, C.M.; Thomas, C.L.; Klinge, D.M.; Bernauer, A.M.; Liu, Y.; Dacic, S.; Siegfried, J.M.; Dahlberg, S.E.; Schiller, J.H.; et al. SULF2 Methylation Is Prognostic for Lung Cancer Survival and Increases Sensitivity to Topoisomerase-I Inhibitors via Induction of ISG15. *Oncogene* **2012**, *31*, 4107–4116. [CrossRef] [PubMed]
47. Wang, J.-M.; Liu, B.-Q.; Zhang, Q.; Hao, L.; Li, C.; Yan, J.; Zhao, F.-Y.; Qiao, H.-Y.; Jiang, J.-Y.; Wang, H.-Q. ISG15 Suppresses Translation of ABCC2 via ISGylation of hnRNPA2B1 and Enhances Drug Sensitivity in Cisplatin Resistant Ovarian Cancer Cells. *Biochim. Biophys. Acta BBA—Mol. Cell Res.* **2020**, *1867*, 118647. [CrossRef] [PubMed]
48. Zhang, Q.; Wang, J.; Qiao, H.; Huyan, L.; Liu, B.; Li, C.; Jiang, J.; Zhao, F.; Wang, H.; Yan, J. *ISG15* Is Downregulated by KLF12 and Implicated in Maintenance of Cancer Stem Cell-like Features in Cisplatin-resistant Ovarian Cancer. *J. Cell. Mol. Med.* **2021**, *25*, 4395–4407. [CrossRef] [PubMed]
49. Wu, S.-F.; Xia, L.; Shi, X.-D.; Dai, Y.-J.; Zhang, W.-N.; Zhao, J.-M.; Zhang, W.; Weng, X.-Q.; Lu, J.; Le, H.-Y.; et al. RIG-I Regulates Myeloid Differentiation by Promoting TRIM25-Mediated ISGylation. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 14395–14404. [CrossRef] [PubMed]

50. Cruz-Ramos, E.; Macías-Silva, M.; Sandoval-Hernández, A.; Tecalco-Cruz, A.C. Non-Muscle Myosin IIA Is Post-Translationally Modified by Interferon-Stimulated Gene 15 in Breast Cancer Cells. *Int. J. Biochem. Cell Biol.* **2019**, *107*, 14–26. [CrossRef] [PubMed]
51. Halim, C.E.; Deng, S.; Crasta, K.C.; Yap, C.T. Interplay Between the Cytoskeleton and DNA Damage Response in Cancer Progression. *Cancers* **2025**, *17*, 1378. [CrossRef] [PubMed]
52. Wardlaw, C.P.; Petrini, J.H.J. ISG15 Conjugation to Proteins on Nascent DNA Mitigates DNA Replication Stress. *Nat. Commun.* **2022**, *13*, 5971. [CrossRef] [PubMed]
53. Chen, Y.-L.; Wu, W.-L.; Jang, C.-W.; Yen, Y.-C.; Wang, S.-H.; Tsai, F.-Y.; Shen, Y.-Y.; Chen, Y.-W. Interferon-Stimulated Gene 15 Modulates Cell Migration by Interacting with Rac1 and Contributes to Lymph Node Metastasis of Oral Squamous Cell Carcinoma Cells. *Oncogene* **2019**, *38*, 4480–4495. [CrossRef]
54. Bolado-Carrancio, A.; Lee, M.; Ewing, A.; Muir, M.; Macleod, K.G.; Gallagher, W.M.; Nguyen, L.K.; Carragher, N.O.; Semple, C.A.; Brunton, V.G.; et al. ISGylation Drives Basal Breast Tumour Progression by Promoting EGFR Recycling and Akt Signalling. *Oncogene* **2021**, *40*, 6235–6247. [CrossRef] [PubMed]
55. Cerikan, B.; Schiebel, E. DOCK6 Inactivation Highlights ISGylation as RHO-GTPase Balancer. *Cell Cycle* **2017**, *16*, 304–305. [CrossRef]
56. Xu, S.; Cao, B.; Xuan, G.; Xu, S.; An, Z.; Zhu, C.; Li, L.; Tang, C. Function and Regulation of Rab GTPases in Cancers. *Cell Biol. Toxicol.* **2024**, *40*, 28. [CrossRef] [PubMed]
57. Burks, J.; Reed, R.E.; Desai, S.D. ISGylation Governs the Oncogenic Function of Ki-Ras in Breast Cancer. *Oncogene* **2014**, *33*, 794–803. [CrossRef] [PubMed]
58. Ribas, A.; Wolchok, J.D. Cancer Immunotherapy Using Checkpoint Blockade. *Science* **2018**, *359*, 1350–1355. [CrossRef] [PubMed]
59. Chen, N.; Fang, W.; Zhan, J.; Hong, S.; Tang, Y.; Kang, S.; Zhang, Y.; He, X.; Zhou, T.; Qin, T.; et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-Driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients with EGFR Mutation. *J. Thorac. Oncol.* **2015**, *10*, 910–923. [CrossRef] [PubMed]
60. Lastwika, K.J.; Wilson, W.; Li, Q.K.; Norris, J.; Xu, H.; Ghazarian, S.R.; Kitagawa, H.; Kawabata, S.; Taube, J.M.; Yao, S.; et al. Control of PD-L1 Expression by Oncogenic Activation of the AKT–mTOR Pathway in Non–Small Cell Lung Cancer. *Cancer Res.* **2016**, *76*, 227–238. [CrossRef]
61. Burks, J.; Fleury, A.; Livingston, S.; Smith, J.P. ISG15 Pathway Knockdown Reverses Pancreatic Cancer Cell Transformation and Decreases Murine Pancreatic Tumor Growth via Downregulation of PDL-1 Expression. *Cancer Immunol. Immunother.* **2019**, *68*, 2029–2039. [CrossRef] [PubMed]
62. Qu, T.; Zhang, W.; Yan, C.; Ren, D.; Wang, Y.; Guo, Y.; Guo, Q.; Wang, J.; Liu, L.; Han, L.; et al. ISG15 Targets Glycosylated PD-L1 and Promotes Its Degradation to Enhance Antitumor Immune Effects in Lung Adenocarcinoma. *J. Transl. Med.* **2023**, *21*, 341. [CrossRef]
63. Luo, W.; Zhang, D.; Lin, Z.; Zhuang, J.; Liang, S.; Huang, Z.; Zhou, C. Interferon-Stimulated Gene Subtypes as Key Indicators of Immune Landscape and Survival Outcomes in Ovarian Cancer. *Discov. Oncol.* **2024**, *15*, 775. [CrossRef]
64. Wei, J.; Zhuang, Y.; Jiang, C.; Chen, L.; Yuan, B.; Zhao, Y.; Li, H.; Mao, J.-H.; Hang, B.; Ye, C.; et al. Cohort-Based Pan-Cancer Analysis and Experimental Studies Reveal ISG15 Gene as a Novel Biomarker for Prognosis and Immunotherapy Efficacy Prediction. *Cancer Immunol. Immunother.* **2025**, *74*, 168. [CrossRef] [PubMed]
65. Caporali, S.; Calabrese, C.; Minieri, M.; Pieri, M.; Tarantino, U.; Marini, M.; D'Ottavio, S.; Angeletti, S.; Mauriello, A.; Cortese, C.; et al. The miR-133a, TPM4 and TAp63γ Role in Myocyte Differentiation Microfilament Remodelling and Colon Cancer Progression. *Int. J. Mol. Sci.* **2021**, *22*, 9818. [CrossRef] [PubMed]
66. Arnal, I.; Wade, R.H. How Does Taxol Stabilize Microtubules? *Curr. Biol.* **1995**, *5*, 900–908. [CrossRef] [PubMed]
67. Škubník, J.; Pavlíčková, V.; Ruml, T.; Rimpelová, S. Current Perspectives on Taxanes: Focus on Their Bioactivity, Delivery and Combination Therapy. *Plants* **2021**, *10*, 569. [CrossRef]
68. Wang, R.C.; Chen, X.; Parissenti, A.M.; Joy, A.A.; Tuszynski, J.; Brindley, D.N.; Wang, Z. Sensitivity of Docetaxel-Resistant MCF-7 Breast Cancer Cells to Microtubule-Destabilizing Agents Including Vinca Alkaloids and Colchicine-Site Binding Agents. *PLoS ONE* **2017**, *12*, e0182400. [CrossRef] [PubMed]
69. Bates, D.; Eastman, A. Microtubule Destabilising Agents: Far More than Just Antimitotic Anticancer Drugs. *Br. J. Clin. Pharmacol.* **2017**, *83*, 255–268. [CrossRef]
70. Moudi, M.; Go, R.; Yien, C.Y.S.; Nazre, M. Vinca Alkaloids. *Int. J. Prev. Med.* **2013**, *4*, 1231–1235.
71. Banyal, A.; Tiwari, S.; Sharma, A.; Chanana, I.; Patel, S.K.S.; Kulshrestha, S.; Kumar, P. Vinca Alkaloids as a Potential Cancer Therapeutics: Recent Update and Future Challenges. *3 Biotech* **2023**, *13*, 211. [CrossRef] [PubMed]
72. Chanez, B.; Gonçalves, A.; Badache, A.; Verdier-Pinard, P. Eribulin Targets a Ch-TOG-Dependent Directed Migration of Cancer Cells. *Oncotarget* **2015**, *6*, 41667–41678. [CrossRef] [PubMed]
73. Helfrich, B.A.; Gao, D.; Bunn, P.A. Eribulin Inhibits the Growth of Small Cell Lung Cancer Cell Lines Alone and with Radiotherapy. *Lung Cancer* **2018**, *118*, 148–154. [CrossRef] [PubMed]

74. Hüsemann, L.C.; Reese, A.; Radine, C.; Piekorz, R.P.; Budach, W.; Sohn, D.; Jänicke, R.U. The Microtubule Targeting Agents Eribulin and Paclitaxel Activate Similar Signaling Pathways and Induce Cell Death Predominantly in a Caspase-Independent Manner. *Cell Cycle* **2020**, *19*, 464–478. [CrossRef] [PubMed]
75. Eslamian, G.; Wilson, C.; Young, R.J. Efficacy of Eribulin in Breast Cancer: A Short Report on the Emerging New Data. *OncoTargets Ther.* **2017**, *10*, 773–779. [CrossRef] [PubMed]
76. Strouhalova, K.; Přechová, M.; Gandalovičová, A.; Brábek, J.; Gregor, M.; Rosel, D. Vimentin Intermediate Filaments as Potential Target for Cancer Treatment. *Cancers* **2020**, *12*, 184. [CrossRef] [PubMed]
77. Čermák, V.; Dostál, V.; Jelínek, M.; Libusová, L.; Kovář, J.; Rösel, D.; Brábek, J. Microtubule-Targeting Agents and Their Impact on Cancer Treatment. *Eur. J. Cell Biol.* **2020**, *99*, 151075. [CrossRef] [PubMed]
78. Tadijan, A.; Samaržija, I.; Humphries, J.D.; Humphries, M.J.; Ambriović-Ristov, A. KANK Family Proteins in Cancer. *Int. J. Biochem. Cell Biol.* **2021**, *131*, 105903. [CrossRef] [PubMed]
79. Kim, H.-R.; Park, J.-S.; Karabulut, H.; Yasmin, F.; Jun, C.-D. Transgelin-2: A Double-Edged Sword in Immunity and Cancer Metastasis. *Front. Cell Dev. Biol.* **2021**, *9*, 606149. [CrossRef] [PubMed]
80. Thompson, O.; Moghraby, J.S.; Ayscough, K.R.; Winder, S.J. Depletion of the Actin Bundling Protein SM22/Transgelin Increases Actin Dynamics and Enhances the Tumourigenic Phenotypes of Cells. *BMC Cell Biol.* **2012**, *13*, 1. [CrossRef] [PubMed]
81. Rosier, M.; Krstulović, A.; Kim, H.R.; Kaur, N.; Enakireru, E.M.; Symmes, D.; Dobra, K.; Chen, R.; Evans, C.A.; Gad, A.K.B. The Vimentin-Targeting Drug ALD-R491 Partially Reverts the Epithelial-to-Mesenchymal Transition and Vimentin Interactome of Lung Cancer Cells. *Cancers* **2024**, *17*, 81. [CrossRef] [PubMed]
82. Bargagna-Mohan, P.; Hamza, A.; Kim, Y.; Khuan Abby Ho, Y.; Mor-Vaknin, N.; Wendschlag, N.; Liu, J.; Evans, R.M.; Markovitz, D.M.; Zhan, C.-G.; et al. The Tumor Inhibitor and Antiangiogenic Agent Withaferin A Targets the Intermediate Filament Protein Vimentin. *Chem. Biol.* **2007**, *14*, 623–634. [CrossRef] [PubMed]
83. Liu, Q.; Cheng, C.; Huang, J.; Yan, W.; Wen, Y.; Liu, Z.; Zhou, B.; Guo, S.; Fang, W. MYH9: A Key Protein Involved in Tumor Progression and Virus-Related Diseases. *Biomed. Pharmacother.* **2024**, *171*, 116118. [CrossRef] [PubMed]
84. Zhang, X.; Yuan, Z.; Zhang, Y.; Yong, S.; Salas-Burgos, A.; Koomen, J.; Olashaw, N.; Parsons, J.T.; Yang, X.-J.; Dent, S.R.; et al. HDAC6 Modulates Cell Motility by Altering the Acetylation Level of Cortactin. *Mol. Cell* **2007**, *27*, 197–213. [CrossRef] [PubMed]
85. Nakashima, H.; Nguyen, T.; Goins, W.F.; Chiocca, E.A. Interferon-Stimulated Gene 15 (ISG15) and ISG15-Linked Proteins Can Associate with Members of the Selective Autophagic Process, Histone Deacetylase 6 (HDAC6) and SQSTM1/P62. *J. Biol. Chem.* **2015**, *290*, 1485–1495. [CrossRef] [PubMed]
86. Hubbert, C.; Guardiola, A.; Shao, R.; Kawaguchi, Y.; Ito, A.; Nixon, A.; Yoshida, M.; Wang, X.-F.; Yao, T.-P. HDAC6 Is a Microtubule-Associated Deacetylase. *Nature* **2002**, *417*, 455–458. [CrossRef] [PubMed]
87. Pham, T.Q.; Robinson, K.; Xu, L.; Pavlova, M.N.; Skapek, S.X.; Chen, E.Y. HDAC6 Promotes Growth, Migration/Invasion, and Self-Renewal of Rhabdomyosarcoma. *Oncogene* **2021**, *40*, 578–591. [CrossRef] [PubMed]
88. Lee, Y.-S.; Lim, K.-H.; Guo, X.; Kawaguchi, Y.; Gao, Y.; Barrientos, T.; Ordentlich, P.; Wang, X.-F.; Counter, C.M.; Yao, T.-P. The Cytoplasmic Deacetylase HDAC6 Is Required for Efficient Oncogenic Tumorigenesis. *Cancer Res.* **2008**, *68*, 7561–7569. [CrossRef] [PubMed]
89. Zhang, Y.; Thery, F.; Wu, N.C.; Luhmann, E.K.; Dussurget, O.; Foecke, M.; Bredow, C.; Jiménez-Fernández, D.; Leandro, K.; Beling, A.; et al. The in Vivo ISGylome Links ISG15 to Metabolic Pathways and Autophagy upon Listeria Monocytogenes Infection. *Nat. Commun.* **2019**, *10*, 5383. [CrossRef] [PubMed]
90. Latario, C.J.; Schoenfeld, L.W.; Howarth, C.L.; Pickrell, L.E.; Begum, F.; Fischer, D.A.; Grbovic-Huezo, O.; Leach, S.D.; Sanchez, Y.; Smith, K.D.; et al. Tumor Microtubes Connect Pancreatic Cancer Cells in an Arp2/3 Complex-Dependent Manner. *Mol. Biol. Cell* **2020**, *31*, 1259–1272. [CrossRef] [PubMed]
91. Zhao, C.; Denison, C.; Huibregtse, J.M.; Gygi, S.; Krug, R.M. Human ISG15 Conjugation Targets Both IFN-Induced and Constitutively Expressed Proteins Functioning in Diverse Cellular Pathways. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10200–10205. [CrossRef] [PubMed]
92. Proudfit, A.; Bhunia, N.; Pore, D.; Parker, Y.; Lindner, D.; Gupta, N. Pharmacologic Inhibition of Ezrin-Radixin-Moesin Phosphorylation Is a Novel Therapeutic Strategy in Rhabdomyosarcoma. *Sarcoma* **2020**, *2020*, 9010496. [CrossRef] [PubMed]
93. Carmeci, C.; Thompson, D.A.; Kuang, W.W.; Lightdale, N.; Furthmayr, H.; Weigel, R.J. Moesin Expression Is Associated with the Estrogen Receptor-Negative Breast Cancer Phenotype. *Surgery* **1998**, *124*, 211–217. [CrossRef]
94. Alam, F.; Mezhal, F.; El Hasasna, H.; Nair, V.A.; Aravind, S.; Saber Ayad, M.; El-Serafi, A.; Abdel-Rahman, W.M. The Role of P53-microRNA 200-Moesin Axis in Invasion and Drug Resistance of Breast Cancer Cells. *Tumor Biol.* **2017**, *39*, 101042831771463. [CrossRef] [PubMed]
95. Takeuchi, T.; Inoue, S.; Yokosawa, H. Identification and Herc5-Mediated ISGylation of Novel Target Proteins. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 473–477. [CrossRef] [PubMed]

96. Wattanathamsan, O.; Thararattanobon, R.; Rodsiri, R.; Chanvorachote, P.; Vinayanuwattikun, C.; Pongrakhananon, V. Tubulin Acetylation Enhances Lung Cancer Resistance to Paclitaxel-Induced Cell Death through Mcl-1 Stabilization. *Cell Death Discov.* **2021**, *7*, 67. [CrossRef] [PubMed]
97. Yue, J.; Lu, H.; Liu, J.; Berwick, M.; Shen, Z. Filamin-A as a Marker and Target for DNA Damage Based Cancer Therapy. *DNA Repair* **2012**, *11*, 192–200. [CrossRef] [PubMed]
98. Honda, K.; Yamada, T.; Hayashida, Y.; Idogawa, M.; Sato, S.; Hasegawa, F.; Ino, Y.; Ono, M.; Hirohashi, S. Actinin-4 Increases Cell Motility and Promotes Lymph Node Metastasis of Colorectal Cancer. *Gastroenterology* **2005**, *128*, 51–62. [CrossRef] [PubMed]
99. Gold, I.M.; Reis, N.; Glaser, F.; Glickman, M.H. Coronaviral PLpro Proteases and the Immunomodulatory Roles of Conjugated versus Free Interferon Stimulated Gene Product-15 (ISG15). *Semin. Cell Dev. Biol.* **2022**, *132*, 16–26. [CrossRef] [PubMed]
100. Bang, W.; Kim, J.; Seo, K.; Lee, J.; Han, J.H.; Park, D.; Cho, J.H.; Shin, D.; Kim, K.-H.; Song, M.J.; et al. Suppression of SARS-CoV-2 Nucleocapsid Protein Dimerization by ISGylation and Its Counteraction by Viral PLpro. *Front. Microbiol.* **2024**, *15*, 1490944. [CrossRef] [PubMed]
101. Rhamadianti, A.F.; Abe, T.; Tanaka, T.; Ono, C.; Katayama, H.; Makino, Y.; Deng, L.; Matsui, C.; Moriishi, K.; Shima, F.; et al. SARS-CoV-2 Papain-like Protease Inhibits ISGylation of the Viral Nucleocapsid Protein to Evade Host Anti-Viral Immunity. *J. Virol.* **2024**, *98*, e0085524. [CrossRef] [PubMed]
102. Bonacci, T.; Emanuele, M.J. DIG-DUBs: Mechanisms and Functions of ISG15 Deconjugation by Human and Viral Cross-Reactive Ubiquitin Proteases. *Biochem. Soc. Trans.* **2025**, *53*, 787–800. [CrossRef] [PubMed]
103. Fu, Z.; Huang, B.; Tang, J.; Liu, S.; Liu, M.; Ye, Y.; Liu, Z.; Xiong, Y.; Zhu, W.; Cao, D.; et al. The Complex Structure of GRL0617 and SARS-CoV-2 PLpro Reveals a Hot Spot for Antiviral Drug Discovery. *Nat. Commun.* **2021**, *12*, 488. [CrossRef] [PubMed]
104. Shan, H.; Liu, J.; Shen, J.; Dai, J.; Xu, G.; Lu, K.; Han, C.; Wang, Y.; Xu, X.; Tong, Y.; et al. Development of Potent and Selective Inhibitors Targeting the Papain-like Protease of SARS-CoV-2. *Cell Chem. Biol.* **2021**, *28*, 855–865.e9. [CrossRef] [PubMed]
105. Al-Homoudi, A.I.; Kushwaha, N.D.; Aruri, H.; Galarza, D.; Moorman, N.J.; Heise, M.T.; Tartamella, J.; Ray, D.; Kovari, L.C.; Gavande, N.S. Structure-Guided Design of Potent and Selective Covalent Inhibitors of SARS-CoV-1 and SARS-CoV-2 Papain-like Proteases with Antiviral Activity. *Eur. J. Med. Chem.* **2026**, *317*, 119108. [CrossRef] [PubMed]
106. Chutoe, C.; Webb, E.R.; Muir, M.T.; Laing, F.; Brunton, V.G.; Kriegsheim, A.V. USP18 Inhibition Enhances Type I Interferon Signalling and Immune Activation in the Tumour Microenvironment of Triple-Negative Breast Cancer 2025. *bioRxiv* **2025**. [CrossRef]
107. Ogunlade, B.O.; Dalby, K.N.; Okpechi, S.C.; Cho, E.J.; Tyutyunyk-Massey, L.; Chen, Z.; Liu, X.; Ivanic, J.; Luke, B.; Desai, S.D.; et al. Derivation and Characterization of Ubiquitin-Specific Protease 18 Inhibitors. *JCI Insight* **2026**, *11*, e202853. [CrossRef] [PubMed]
108. Wood, L.M.; Pan, Z.-K.; Seavey, M.M.; Muthukumaran, G.; Paterson, Y. The Ubiquitin-like Protein, ISG15, Is a Novel Tumor-Associated Antigen for Cancer Immunotherapy. *Cancer Immunol. Immunother.* **2012**, *61*, 689–700. [CrossRef] [PubMed]
109. Nguyen, H.-M.; Oladejo, M.; Paulishak, W.; Wood, L.M. A Listeria-Based Vaccine Targeting ISG15 Exerts Anti-Tumor Efficacy in Renal Cell Carcinoma. *Cancer Immunol. Immunother.* **2023**, *72*, 2889–2903. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.