REVIEW ARTICLE

Cancer Cells Exploit Sublethal Apoptotic Signaling as an Adaptive Mechanism to Evade Immune Surveillance and Drive Relapse

Abdelalim Y. Abusabha[1], Maysa Alhawamdeh[2], Saeed A. Al Buasedah[1], Saqer M. Alhammadi[1], Rami J. Alshehri[1], Azzam M. Alwadai[1], Fahad H. Al Zamanan[1], Muhannad A. Alyami[1], Mohamed O. Mohamed[3], Sara A. Mekkawy[4], Mona I. Shahin[5], Mohamed R. Eletmany[6*]

[1]Research Sector, Dar Al-Ahfad Academy for Education, Al Athaybah District, Najran 66258, Saudi Arabia. Emails: abdelalimabusabha@gmail.com (A.Y.A.); Salwla29@gmail.com (S.A.A.); aljbyrymtb507@gmail.com (S.M.A.); r05046127@icloud.com (R.J.A.); Aaazzam811@gmail.com (A.M.A.); ffahad23444@gmail.com (F.H.A.); hamad.mb2025@icloud.com (M.A.A.)

[2]Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Mutah University, Al Karak 61710, Jordan. Email: maysa5005@mutah.edu.jo, ORCID: https://orcid.org/0000-0001-9215-7643

[3]Ain Shams University, Faculty of Agriculture, Agriculture Biotechnology Program, P.O. Box 68, Hadyek Shoubra, 11241, Cairo, Egypt. Email: mohamadmasr678@gamil.com

[4]Molecular Biotechnology program, Chemistry Department, Faculty of Science, Capital University (Formerly Helwan University), 11795, Cairo, Egypt. Email: Saraahmed9572_892@science.helwan.edu.eg

[5]Biology department, Faculty of Tymaa, Tabuk University, Tymaa 71491, KSA. Email: m.eshaheen@ut.edu.sa

[6*]Chemistry Department, Faculty of Science, Qena University, Qena 83523, Egypt. Email: mohamed.eletmany@sci.svu.edu.eg; ORCID: https://orcid.org/0000-0003-4868-4678 (Corresponding Author)

ABSTRACT

Oncogene-targeted therapies often result in significant early tumor reductions; nevertheless, they are eventually constrained by relapse due to minimum residual disease (MRD). This review presents new evidence demonstrating a paradigm shift in the understanding of treatment resistance, with a focus on the non-apoptotic, adaptive roles of apoptotic proteins in cancer persister cells. We demonstrate the mechanisms through which sub-lethal apoptotic signalling in this temporary, drug-tolerant population is utilised for the survival and proliferation of these cells. An important finding is that caspases can activate the DNA fragmentation factor B (DFFB) as a dual mechanism. A model is proposed in which sublethal apoptotic signalling within persister cancer cells activates DFFB, then potentially induces ATF3 that leads to inhibition of type I interferon signalling and promotes tumour regrowth. DFFB activation is achieved by upregulation of activation transcription factor 3 (ATF3) during early stages of stress. The identification of a connection between therapy-induced genotoxic stress and the immune escape mechanism is provided by the DFFB-ATF3 molecular network. This allows persister cells to avoid dormancy caused by IFN production. We have changed the known view of DFFB from a molecule that causes cell death to a key regulator of an adaptive, pro-survival response that leads to eventual relapse. Thus, focusing on this axis offers a viable treatment strategy to eliminate minimal residual disease by concurrently blocking adaptive mutagenesis and reinstating intrinsic immune-mediated growth regulation, providing an innovative combinatorial approach to avert relapse and enhance long-term outcomes.

Keywords: Tumor Relapse, Type I interferon (IFN), Minimal residual disease (MRD), DNA Fragmentation Factor B (DFFB), Cancer Persistence.

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List of abbreviation

ALK	Anaplastic Lymphoma Kinase
AP1	Activator Protein 1

APOBEC	Apolipoprotein B mRNA Editing Enzyme Catalytic Polypeptide-like
ATM	Ataxia Telangiectasia Mutated
ATF3	Activating Transcription Factor 3
ATR	Ataxia Telangiectasia and Rad3-related
BAX	BCL-2 Associated X Protein
BAK	BCL-2 Antagonist/Killer
BAD	BCL-2 Associated Agonist of Cell Death
BCL-2	B-cell Lymphoma 2
BCL-xL	B-cell Lymphoma-extra Large
BRAF	v-Raf Murine Sarcoma Viral Oncogene Homolog B
CAD	Caspase-Activated DNase
cfDNA	Cell-free DNA
CHIP	Clonal Hematopoiesis of Indeterminate Potential
CIA	Caspase-Independent Apoptosis
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
ctDNA	Circulating Tumor DNA
DAMPs	Damage-Associated Molecular Patterns
DDR	DNA Damage Response
DFFB	DNA Fragmentation Factor Subunit B (also CAD)
DFFA	DNA Fragmentation Factor Subunit A (ICAD)
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial-Mesenchymal Transition
FDA	Food and Drug Administration
HER2	Human Epidermal Growth Factor Receptor 2
ICAD	Inhibitor of CAD (DFFA)
ICD	Immunogenic Cell Death
IFN	Interferon
IFN-α	Interferon-alpha
IFN-β	Interferon-beta
IFNAR	Interferon Alpha/Beta Receptor
IRDS	Interferon-Related DNA Damage Resistance Signature
IRF	Interferon Regulatory Factor
ISG	Interferon-Stimulated Gene
JAK	Janus Kinase
MAVS	Mitochondrial Anti-Viral Signaling Protein
MAPK	Mitogen-Activated Protein Kinase
MRD	Minimal Residual Disease
MYC	Myelocytomatosis Oncogene
NGS	Next-Generation Sequencing
NK cell	Natural Killer Cell
NLRP3	NOD-, LRR- and Pyrin Domain-Containing Protein 3
PAMPs	Pathogen-Associated Molecular Patterns

PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
PRR	Pattern Recognition Receptor
PROTACs	Proteolysis-Targeting Chimeras
RAS	Rat Sarcoma Virus Oncogene
RIG-I	Retinoic Acid-Inducible Gene I
SMAC	Second Mitochondria-derived Activator of Caspases
SOCS	Suppressor of Cytokine Signaling
STAT	Signal Transducer and Activator of Transcription
STING	Stimulator of Interferon Genes
TAM	Tumor-Associated Macrophage
TBK1	TANK-Binding Kinase 1
TIM-3	T-cell Immunoglobulin and Mucin-domain containing-3
TME	Tumor Microenvironment
TP53	Tumor Protein p53
TTFields	Tumor Treating Fields
WBC	White Blood Cells

1. Introduction

Oncogene-targeted cancer therapies can afford deep responses but frequently suffer from acquired resistance [1]. Multiple co-occurring resistance mechanisms and ongoing tumor progression challenge therapeutic methods for treating tumors that have developed medication resistance. It may be able to avoid resistance by blocking the adaptive mechanisms that lead to resistance rather than treating it after it manifests, albeit these mechanisms are not well understood [2, 3]. The report investigated that residual cancer persister cells, which survive oncogene-targeted therapy, are growth-arrested due to drug stress-induced intrinsic Type I interferon (IFN) signaling. Persister cells utilize apoptotic mechanisms to transcriptionally inhibit interferon-stimulated genes (ISGs) to overcome growth arrest [1, 4]. Persister cells engage apoptotic caspases in a sublethal manner to activate the DNA endonuclease DNA Fragmentation Factor B (DFFB, or Caspase-Activated DNase, CAD). This activation leads to DNA damage, mutagenesis, and the induction of the stress response factor Activating Transcription Factor 3 (ATF3). Researchers have discovered that ATF3 acts as a suppressor of the expression of endogenous interferon-stimulated genes (ISGs) by inhibiting the activity of Activator Protein-1 (AP1) in the cell. This suppression allows for the development of new persister cells. Persister cells have higher levels of ISGs when compared to those expressing neither DFFB nor ATF3, and cannot reproduce themselves [1, 5]. Therefore, sublethal apoptotic stress unexpectedly confirms the cancer cells' regrowth that survives drug treatment.

1.1. The Challenge of Tumor Relapse and Minimal Residual Disease

Minimal Residual Disease (MRD) refers to the presence of very low levels of cancer cells in a patient during and/or after cancer treatment. The level of residual cancer in a patient, or tumor burden, will vary based on the type and stage of the cancer and the type of treatment received. As a result of the decrease in tumor burden due to the response to treatment, many tumors become "invisible" through imaging or clinical observation. However, if any residual tumor cells are remaining in the body after treatment, these cells can cause relapse of cancer locally or through distant metastasis [6, 7]. The need to detect MRD has been primarily focused on hematologic malignancies because MRD provides oncologists with a means of assessing the efficacy of treatment and predicting both early and late relapse rates for patients. The detection of MRD in solid tumors has allowed for better patient stratification into high vs. low relapse risk categories [8]. As a result, numerous techniques are currently being investigated to accurately detect MRD in solid tumor patients and incorporate MRD detection in the treatment plan [9].

Identifying an MRD would be beneficial for both patients and health care providers. When the standard curative therapy is completed, then treatment can be adjusted according to the results of the liquid biopsy test. If patients who test positive for MRD can have their treatment intensified, this may lead to improved

disease-free survival and overall survival if the MRD is adequately treated. On the other hand, de-escalation strategies (removing adjuvant therapies such as systemic chemotherapy) in patients who are MRD-negative may help reduce patient burden, treatment-related adverse events, increase quality of life and reduce societal economic costs with no reduction in survival [9, 10].

The current clinical study will explore the importance of detecting MRD in guiding subsequent treatment decisions and will include trials for all solid tumor types [11-13]. This strategy relies heavily upon accurately identifying MRD, which cannot currently be assessed via standard imaging techniques or clinical evaluations. A liquid biopsy is one potential way to overcome this limitation. As there is still no agreement on the methods to use when using liquid biopsy to detect MRD, research has been increasing in this field and there have been various advances in the technology and analysis of liquid biopsy technology in the past few years [14, 15].

There was limited gene copy number in the plasma samples, which limited detection for MRD to only a few specific variants, which is the result of this limitation. For typical MRD detection, high sequencing depth is necessary [16, 17]. The ability to detect ctDNA is limited by how sensitive the detection method is. As the frequency of the variant allele gets closer to the limit of detection, it can become increasingly difficult to determine which variants exist in the sample taken. Different cancers have varying fractions of cfDNA as tumors, and the differences between patients with the same type of cancer also vary considerably. Several studies utilizing ctDNA as a measurement indicate that the presence of micro-metastases results in a much larger amount of ctDNA shed from the body compared with the presence of small amounts of residual local disease. For these reasons, it is not possible to avoid some false negatives from ctDNA testing due to the biological features of the cancer, such as a low rate of DNA shed from certain locations or types of metastases. Moreover, the sensitivity of different mutation types is often very different from one another [18, 19]. While different methods have different strengths/weaknesses for detecting different kinds of DNA mutations (single nucleotide variants vs fusions vs copy number alterations), ctDNA only has some degree of sensitivity for identifying structural/copy number alterations in patients. Additionally, when evaluating ctDNA, it is important to keep in mind that if insufficient amounts of ctDNA are present, structural/copy number alterations may not be detectable [20, 21].

False positive results for circulating tumor DNA (ctDNA) may arise due to the presence of DNA fragments from clonal hematopoiesis of indeterminate potential (CHIP) or non-neoplastic hematopoietic stem cells; however, this can be reduced by utilizing advanced bioinformatics methods to analyze ctDNA or through comparison with sequencing data from leukocytes and/or matched tumor samples. Identifying CHIP mutations remains a potential source of misinterpretation when assessing tumor mutations without having access to WBC reference samples. As a result, it is suggested that further Next Generation Sequencing (NGS) analysis of leukocytes be conducted in order to eliminate CHIP-related mutations, especially in cases of MRD or early detection of cancer [22, 23].

The concordance between ctDNA and tissue-based NGS data is defined by whether or not both molecular platforms reveal the same genomic alterations of a specific gene. Many factors contribute to unequal detection of ctDNA and tissue, including the location and timing of the biopsy as well as the variability of DNA shed by a tumor, differences in tumor heterogeneity and differences in epigenetic control. The lack of consistency seen among different testing modalities for ctDNA offers an additional barrier for the user in interpreting obtained results. Factors that may contribute to inconsistent testing of ctDNA include the timing of blood sample collection, blood collection method, method of sample storage, library preparation technique, method for assigning unique molecular IDs to ctDNA, and bioinformatic analysis of data [24, 25].

Assessment of risk and the administration of adjuvant treatment are imperative factors in treating cancer patients. The ability to identify people with MRD with ctDNA analysis allows for the identification of those patients who would benefit from adjuvant chemotherapy as well as eliminating those patients from receiving the unnecessary treatment. By determining the length of time a patient will need adjuvant therapy based upon whether they clear the ctDNA, and hence the potential for adverse effects, researchers are able to reduce the likelihood of a harmful event occurring. This is especially important because the standardization of ctDNA testing methods is still quite variable, and therefore it would be inappropriate to exclude patients from receiving adjuvant therapy solely based upon negative ctDNA test results [26, 27].

The initial results from clinical studies that utilize ctDNA for detecting MRD look encouraging, but there are a number of limitations to these studies as most included sample sizes were very small and thus need to be confirmed with large independent patient populations to validate these findings. Therefore, additional research is required to answer the important question of whether the use of ctDNA MRD can lead

to improved clinical outcomes with subsequent interventions and whether the use of ctDNA MRD can assist to better identify patients who would benefit from adjuvant therapy [28, 29].

1.2. Apoptotic Proteins Beyond Cell Death: Emerging Non-Canonical Roles

Many studies over the past several years have revealed different types of non-apoptotic cell death in many organisms such as Caenorhabditis elegans, Drosophila melanogaster, humans and many other multicellular organisms [30]. These mechanisms of cell death are known as "non-canonical" because they are different from the conventional apoptotic process, which uses caspases to induce apoptosis (programmed cell death). The non-canonical pathways include CIA (caspase-independent apoptosis), ferroptosis, necrosis, autophagy, mitotic catastrophe, paraptosis and pyroptosis. They differ with respect to their causes, morphological characteristics, and the proteins or pathways involved [31, 32]. Therefore, many non-apoptotic, non-canonical cell pathways are independent of caspases; they may provide an alternate route for inducing cell death when apoptosis fails [32, 33].

The most frequently mutated tumor suppressor in human cancers is p53, which functions as a tumor suppressor through a variety of means including regulating the transcription of many genes involved in the cell cycle and apoptosis through its function as a transcription factor [34, 35]. In response to both internal (e.g. oxidative stress) and external (e.g. DNA damage) signals, p53 becomes stabilized and becomes active, primarily through the transactivation of genes that promote apoptosis (e.g. BAX, BAD, or Bak). In addition to transactivating these pro-apoptotic genes, p53 can directly interact with several anti-apoptotic mitochondrial proteins, including Bcl-2 or Bcl-xL, to induce apoptosis. Apoptosis induced by p53 is important in the capability of p53 to suppress tumors and make cells more sensitive to treatments such as radiation and chemotherapy [36-38]. Recent research has shown that p53 continues to play a role in non-canonical pathways; however, data suggest that the regulation of interactions between tumor microenvironment and the regulation of metabolic flexibility are critical to p53 in an anti-cancer capacity. Thus, understanding the role that p53 may play in DFFB (DFFB) will aid in understanding its effects on the immune system and the development and progression of cancer. Therefore, targeting the DFFB and ATF3 pathways may represent a viable approach to preventing relapse and addressing MRD in patients with cancer. DFFB activates DFFB in persister cells through apoptosis caspases, thereby inducing continuous DNA damage, blocking the action of growth-arresting interferon signals, and allowing persister cells to bypass their normal growth this way to develop tumour that are drug-resistant. The goal of inhibiting DFFB and ATF3 is to disable adaptive mechanisms, which may enhance the efficacy of targeted therapies and increase the survival of patients [39, 40].

1.3. Type I Interferon Signaling as a Guardian Against Cancer Persistence

Tumor development often occurs in environments characterized by chronic inflammation and immunologic activity. Although transformed cells can be recognized by the immune system and subsequently eliminated, tumors can develop ways of evading regulation from the immune system by changing their microenvironment. A key mediator of this escape route is macrophages [41, 42]. Macrophages play a crucial role, initially performing anti-tumor functions before transitioning to a tumor-associated phenotype that suppresses anti-tumor immune responses and contributes to a persistent inflammatory, growth-promoting tumor microenvironment (TME) [43, 44]. Macrophages initially play an anti-tumor role before adopting a phenotype that is associated with the tumor, thus inhibiting the anti-tumor immune response while establishing a tumor microenvironment that supports inflammation and growth. Type I interferons (IFNs) are known to regulate inflammatory responses significantly. In addition to their activity to inhibit tumor growth directly, IFNs alter the function of immune cells in the tumor microenvironment (TME) (see Figure 1) [45, 46]. Tumor-intrinsic determinants of IFN-stimulated gene (ISG) expression include nucleic acids, metabolites, and hypoxia. This review will discuss how IFN-induced changes in macrophage phenotype impact differentiation, polarization, and function. Finally, we will discuss how macrophages mediate tumor cell killing and phagocytosis, as well as how they affect their environment through cytokine release and interaction with immune cells [47, 48].

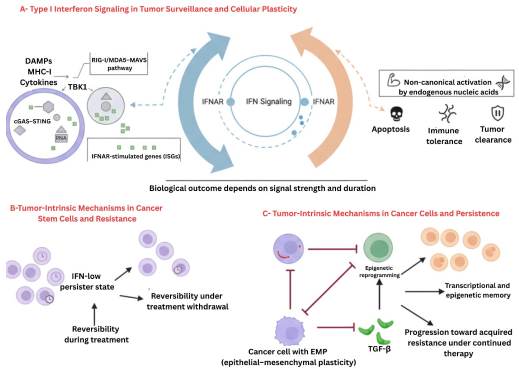

Figure 1: Type I interferon signaling regulates tumor surveillance, cellular plasticity, and therapy resistance through tumor-intrinsic mechanisms. A- Type I interferon signaling in tumor surveillance, cellular

plasticity. B- Tumor-intrinsic mechanisms in cancer stem cells and resistance. C- Tumor-intrinsic mechanisms in cancer cells and persistence.

Type I interferons primarily induce anti-tumorigenic effects in monocytes and macrophages within the tumor microenvironment. Type I IFN responses in myeloid cells inhibit the differentiation of monocytes into tumor-associated macrophages (TAMs), promote pro-inflammatory polarization, enhance the killing of tumor cells, and positively influence the anti-tumor functions of other immune cells, particularly T cells, during their interactions with macrophages. Various tumor-intrinsic characteristics promote the production of type I interferons; however, the resultant selection pressure compels tumors to develop adaptive strategies to diminish or inhibit type I interferon production.

Elevated concentrations of type I IFNs in the TME frequently correlate with improved prognosis and outcomes. Consequently, direct administration of type I IFNs, stimulation of endogenous type I IFN production, and selective induction of specific interferon-stimulated gene (ISG) subsets have emerged as promising therapeutic strategies for tumors, as illustrated in Figure 2 [49, 50]. Administration of exogenous type I IFNs as alternative or adjuvant tumor therapy has been primarily evaluated based on their effects on other immune cells and their cytostatic impact on tumor cells [51]. IFN-α2a and IFN-α2b were approved for use in the clinic; however, numerous studies are still ongoing to study the effectiveness of combining IFN-α with either chemotherapy or immune checkpoint blockade therapy. Systemic administration of recombinant type I interferons often results in considerable toxicity and the development of auto-immunities [52, 53]. The therapeutic efficacy of STING agonists has been tested in early clinical trials, yet their toxicity profiles are still under investigation [54, 55]. Therefore, an alternative approach would be to limit the targeting of individual IFN responses to macrophages in the tumor microenvironment, so as to amplify or restore their anti-tumor activity without the adverse side effects associated with broadly targeting the system [56]. In addition to the direct administration of recombinant type I interferons, many novel therapies have also been shown to be able to promote IFN production [57]. Therapies that can elicit an "Immunogenic" cell death (ICD) response are associated with a unique kind of "Programmed" Cell Death that Activates the Immune System" through an increase in the amount of type I IFNs produced. This is exhibited through the Quantification of type 1 IFNs, which serve as a Biomarker for successfully Inducing Immunogenic Cell Death [58, 59]. Most of the currently available ICD inducers fall under the medicinal, radiotherapeutic, or targeted anticancer Agent categories. The release of DAMPs, either from within cells or to other parts of the body, triggers or potentiates the type 1 IFN response from certain cells in the innate immune response (particularly Monocytes and Macrophages) that have PRRs particularly elevated in their expression [60, 61].

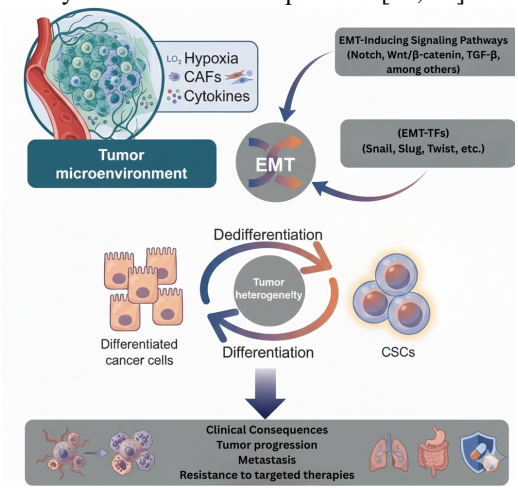

Figure 2: The role of the tumor microenvironment in driving epithelial–mesenchymal transition (EMT) and cancer cell plasticity.

Following radiotherapy, tumor cells and hematopoietic cells generate type I interferons, likely as a result of detecting double-stranded DNA from irradiated tumor cells or due to micronuclei that arise during mitotic progression after radiotherapy-induced double-stranded DNA breaks. Therapy utilizing Tumor Treating Fields (TTFields), an FDA-approved treatment for glioblastoma and malignant mesothelioma, applies low-energy alternating electric fields that disrupt mitosis and lead to micronuclei formation. This approach has been demonstrated to induce type I interferons and interferon-stimulated genes [62, 63]. Type I interferons seem to play a role in facilitating anti-tumor immune responses in both scenarios. While the mechanisms explained in the first part of this review lead primarily to moderate levels of type I interferon (IFN). Still, many oncologists may assume that therapies that induce cellular death will greatly increase levels of type I IFN due to the massive amount of self-nucleic acids and other damage associated molecules (DAMPs) released into the post-therapeutic environment. It is also essential to realize that while the physiological induction of type I IFN is considered anti-tumorigenic, when sustained and elevated levels of type I IFN are found in tumour environments, they cause increased expression of a group of IFN-stimulated genes (ISGs) known as the “IFN-related DNA damage resistance signature” (IRDS). This is implicated in the development of resistance to treatment [46, 64].

Therapeutic intervention can rapidly and greatly stimulate the production of Type I IFNs and as such, the properties of Type I IFNs in relation to anti-tumor effects are enhanced. For an extensive review of how Type I IFN levels impact the ratio between pro- and anti-tumor effects. The IRDS represented in tumors corresponds with the mechanism of action of the antiviral response as mediated by increased production of STAT1/2 and IRF9 proteins, which enable the induction of certain ISGs even in the absence of phosphorylation [45, 47]. It has been partially understood the mechanism by which IRDS are induced in tumors; however, it can be theorized that similar resistance mechanisms may be exhibited by macrophages that have been chronically exposed to low concentrations of Type I IFNs. In this regard, the modulation of macrophage differentiation and activation resulting from chronic exposure to Type I IFNs would potentially inhibit the effect of pro-inflammatory polarization and would stimulate an increase in the Anti-tumorigenic functions of macrophages, resulting from acute Type I IFN stimulation. The enhancement of the therapeutic efficacy of Type I IFN stimulated macrophages may be the result of the restoration of Type I IFN responses in myeloid cells through the 'counteraction' of tumor immune escape mechanisms that mediate the down-regulation of receptor sensing pathways and or cause the degradation of nucleic acids. Factors that are present in the tumor microenvironment, such as hypoxia and metabolic intermediate compounds, modulate Type I IFN responses in a cellular type specific manner and such specificity may be exploited for the purpose of enhancing the Anti-tumorigenic functional activities of macrophages that are IFN dependent [65, 66].

1.4. Conceptual Framework: DFFB as a Molecular Nexus in Therapy Resistance and Immune Evasion

Tumor adaptation resulting in acquired drug resistance during oncogene-targeted therapy greatly reduces the efficacy of these therapies, which can directly contribute to patient mortality [67]. There is insufficient characterisation of the processes/residual surviving cancer cells utilise to adapt to this therapeutic stress. Understanding these processes may enable the development of innovative strategies to prevent recurrences of tumors. We also utilised cell culture and mouse xenograft models to evaluate how long-term treatment with targeted oncogene therapy engages surviving "persister" cells to create new colonies and how this behaviour mimics acquired drug resistance [68]. Such persister cells could potentially be those that utilize sublethal apoptotic signaling, as described by in vitro EGFR-mutant lung cancer models where caspase- activated DNase DFFB is thought to enhance the regrowth potential under drug pressure [69, 70]. The results demonstrate that while DFFB WT persister cells created colonies in the presence of the drug, DFFB-deficient persister cells showed a significant reduction in their ability to regrow [71-73]. The ability of DFFB to inflict continuous DNA damage would also lead to mutagenesis and the blocking of growth-arresting interferon signalling [74]. When a cell is receiving stress from drugs, permeability of the mitochondrial outer membrane happens in conjunction with activating a pattern recognition receptor (PRR) that begins signaling for interferons. In addition, the RNA sequencing analysis gathered through single-cell analysis shows that DFFB NULL SUDs show higher expression of genes associated with IFN signalling when compared to Wild Type.

DFFB-Knockout Cell Lines Expressed Higher Levels of STAT1 & STAT2 and Increased Interferon Signaling and Colony Formation Compared to Untreated DFFB-KO Cell Lines Transiently Treated with JAK1/2 Inhibitors [75]. The Transcription Factor ATF3 Is Induced by Stressors Found Only in DFFB-Wild-Type Cell Lines; ATF3 Is Also Induced by DNA Damage Stress and Is Expressed at High Levels in Tumors of Cancer Patients with MRD being Treated with Targeted Therapy [76, 77]. Consistent with the ATF3's ability to Suppress Interferon Signaling, Depletion of ATF3 in Persister Cells Resulted in an Increase in STAT1 Expression Levels, While Over-Expressing ATF3 in DFFB-KO Persistent Tumor Cells Decreased Levels of STAT1. Taken together, these findings suggest a model where DNA damage caused by DFFB expression could lead to the induction of ATF3 with potential consequences of interferon suppression and promotion of regrowth. While the mechanism of DFFB → ATF3 → IFN appears important genetically, this path was not directly proved [78]. Patients' survival rates and the effectiveness of targeted therapies will likely improve as a result of this new method of tumour adaptation; it's a potential means of enhancing the effectiveness of existing therapies. DFFB is non-essential for viability, as knockout mice for DFFB are found to be fertile and grossly normal [79], but display context-dependent immunological defects in the function of immune cells and apoptotic DNA fragmentation. Thus, for example, DFFB-deficient lysates display defective chromatin breakdown and immune responses [80], whereas an impaired elimination of apoptotic DNA contributes to autoimmune inflammation [81]. These data thus implicate DFFB as an immunological factor in defining the quality of apoptotic cell death—the issues also being relevant for tumor-immune cross-communications [82, 83]. Additionally, high levels of DFFB and ATF3 are evidence of poor survival rates in

patients. Thus, DFFB has the potential to be a promising option to overcome drug resistance associated with cancer treatment [74]. The context-specific phenotypes of DFFB deficiency in mice make it particularly relevant to cancer treatments. Defects in apoptotic DNA fragment breaks might influence the immunogenicity of dying cancer cells, which could have an effect on anti-tumor immunity [84, 85]. In addition, defects in interferon or immune cells in DFFB-deficient mice might have a permissive environment for persister cell survival. Therefore, although DFFB is a non-essential gene for viability, it could have important effects on cancer persistence and relapse based on its role in regulating immunity and cell death quality during cancer treatments [86, 87].

Although the DFFB/ATF3/interferon pathway represents an appealing hypothesis for persister cell adaptation, there are some essential validation steps required before applying this hypothesis generally. Firstly, epistasis experiments, like ATF3 overexpression in DFFB knockout mice, are required to validate direct causality. Secondly, the relevance of this pathway to different oncogenic models like BRAF, ALK, and HER2, as well as different tissue types, remains poorly investigated. Thirdly, in vivo relapse models showing that targeting DFFB/ATF3 can slow down relapses would improve the translational validity of this pathway. Future studies should also determine whether this pathway is applicable in hematologic malignancies or whether it specifically focuses on solid malignancies.

2. Non-Canonical Functions of Apoptotic Proteins in Cancer

2.1. Overview of Apoptotic Protein Repurposing

Apoptotic proteins were historically viewed as exclusive mediators of programmed cell death. New evidence in tumors, however, shows that core components of apoptotic proteins are often activated at sub-lethal levels, and are used to help cancer cells survive, adapt, and evolve. Rather than irreversibly committing cells to death, apoptotic proteins can operate along a quantitative continuum, where partial or spatially restricted activation generates adaptive signaling outputs without triggering terminal apoptosis. Depending on the identity and extent of activation of specific apoptotic components, cellular viability may be preserved despite engagement of traditionally lethal pathways. This phenomenon is commonly referred to as sub-lethal or non-canonical apoptotic signaling [88, 89].

This ability of cancer cells to adapt and change to their surroundings and evolving environment is a result of the way apoptotic proteins work. There are several components to apoptotic proteins, all of which have their own ways of working. Upstream factors can turn on proteins, and components which are activated downstream can start the cellular death [90, 91]. The cancer cells exploit these proteins to their own advantage. The proteins are still able to function, and cancer cells can use these functions to adapt to their new environment and gain new benefits, all while avoiding cellular death themselves. This signaling is not merely a passive response to stress, but is increasingly recognized as a contributory mechanism supporting tumor adaptation and evolutionary fitness [92, 93].

Apoptotic proteins include DNA fragmentation factor subunit B (DFFB) also known as CAD. DFFB was previously thought to be a terminal nuclease solely responsible for inducing inter-nucleosomal DNA fragmentation during apoptosis. It is now known that in cancer cells, DFFB functions as a regulator of DNA damage responses, inflammation, and immune evasion. DFFB exemplifies a rheostat-like mode of apoptotic protein function, rather than a binary on–off switch, thereby expanding the conceptual framework through which apoptotic signaling influences tumor fate [94].

2.2. DFFB: From DNA Fragmentation to DNA Damage Response

DFFB is accepted to work only after active execution caspase-3 is present, and it is freed from lisencing inhibition by ICAD, which allows for rapid and widespread fragmentation of chromosomal DNA during apoptosis. Recent studies suggest that DFFB can be activated at sub-lethal levels downstream of caspase-3, resulting in limited and regulated DNA fragmentation without full apoptotic execution, and that DFFB can cause the discernible, regulated fragmentation of chromosomal DNA, and paradoxically allowing the living, cancer host cell to survive instead of die. It is especially observed in cancer cells and during targeted therapeutics, where active, apoptotic, die signals cause a pause in death of the cancer cells [95, 96].

Williams *et al.* demonstrated that cancer persister cells retain sustained, low-level DFFB activity in the absence of full apoptotic commitment, accompanied by sub-apoptotic caspase-3 activation, This DFFB activity was associated with persistent engagement of ATM- and ATR-mediated DNA damage response pathways, indicating ongoing but tolerable genotoxic stress [97]. The persistence of activation of DFFB, which keeps the two DDR pro-apoptotic signals engaged Sustained DFFB-driven genotoxic stress, promoting repeated cycles of DNA

damage and repair, increasing genomic heterogeneity without inducing catastrophic cell death, and thus a sub-population of cells escapes/evades a drug, which is a phenotypic mutation of the population containing the drug. Importantly, this is observed in different cancer cell sub-populations, initiating the cellular defense response, and DFFB- DNA damage indicates it is in a different cell cycle accommodating repairs responding to stress, and it is a cellular adaptive hypothesis of a DFFB- response repair to stress designed to repair, not an adaptation hypothesis for a response to DFFB- induced an injury [98].

In addition to its impact on genome fragmentation, DFFB also has an impact on inflammation and immune signaling as well. Moeed *et al.* reported that caspase-activated DNase activity can generate immunostimulatory DNA fragments capable of engaging innate immune sensing pathways and activate programmed responses associated with the defense of the virus [95, 99], and this involves DFFB as DNA fragments that are influential as immunomodulators. In the case of cancer, however, this mechanism appears to be under specific rewiring. A recent study showed that DFFB suppression of type I interferon signaling in immune evading cancer persister cells facilitates their regrowth and immune evasion after therapy [75, 100]. These findings position DFFB as a dual-function effector that both intersects with innate immune sensing pathways and, paradoxically, suppresses type I interferon signaling to facilitate immune evasion; to promote the activation of innate immune sensors with the DNA that is released from tumor cells, while simultaneously using that mechanism to silence the interferon response and avoid immune detection [95].

What has been described above places DFFB in the convergence of DNA damage, inflammation and immune modulation. The significance of these events does not solely classify DFFB as a dismissive loss and loss-of-dominance nuclease, it defines DFFB as an adaptive signaling mechanism and in addition to its role as the end product of this type of execution, DFFB also gives DFFB the ability to transform low levels of apoptotic signals into long periods of positive survival by enhancing the lifespan of cells. The evolution of tumor cells has also been profoundly affected by viewing fragmentation not as a final outcome but rather as a biological signal of evolution that can drive cellular division in response to changes in the surrounding environment [101, 102].

2.3. Other Apoptotic Proteins with Non-Apoptotic Functions

The identification of the repurposing for DFFB indicates a generality that applies to many families of apoptotic proteins. Specifically, the role of caspases in non-lethal stress response has increased in positive appraisal. It has been demonstrated that both caspase-3 and caspase-7 provide cytoprotection via autophagy and permit cell-survival by facilitating nuclear DNA repair when cells are exposed to stressors such as genotoxic or metabolic stress [95, 103]. Overall, these findings contradict previous notions that the executioner caspases are permanently destructive (i.e., signalling irreversible death) and instead view them as important modulators of cellular resilience.

The belief that non-apoptotic caspase signaling plays a role in communication between tumors and surrounding tissue, as well as stimulating the proliferation of nearby cells through paracrine signaling is established. In addition to its ability to aid in tissue regeneration, paracrine caspase-3 activity assists in the repopulation of tumors after they've been treated [104]. More recent studies extend this idea via demonstrating that caspase-three can actively promote oncogenic transformation thru EndoG-dependent activation of Src–STAT3 signaling pathways [105, 106]. Together, these findings highlight a paradoxical role for caspases as facilitators of tumor growth and malignant progression.

BCL-2 circle of relatives proteins in addition exemplify functional diversification past apoptosis regulation. While exceptional recognized for controlling mitochondrial outer membrane permeabilization, a couple of BCL-2 contributors take part in metabolic regulation, mitochondrial dynamics, and necrotic mobile dying pathways. These non-canonical activities allow cancer cells to first-rate-music energy manufacturing and redox stability, in particular beneath hypoxic or nutrient-confined conditions. Importantly, such capabilities might also make a contribution to healing resistance independently of classical apoptotic blockade [107, 108].

Similarly, SMAC/Diablo, historically regarded as an antagonist of inhibitor of apoptosis proteins (IAPs), reveals non-apoptotic roles in irritation and immune modulation. Evidence shows that SMAC/Diablo may contribute to the survival of tumors, chronic infections, and immunosuppression in lung cancers which point to its potential as a future target for therapeutic intervention beyond just an apoptotic sensitizing agent [109, 110]. The results support the notion that mitochondrial proteins involved in apoptosis have multiple functional roles in regulating the interactions between tumors and the immune system.

2.4. Mechanisms of Functional Switching in Tumor Cells

The discovery that apoptotic proteins can also perform non-canonical functions presents a mechanistic challenge for understanding how tumor cells switch from lethal to adaptive responses. A number of non-mutually exclusive proposals have been made regarding how this switch occurs. First, regulating the level of activation is vital; when activated below threshold levels (subthreshold) caspases or nucleases, they can activate signaling pathways without necessarily triggering all the downstream effector pathways involved in apoptosis. Second, the spatial compartmentalisation of substrates restricts substrate access to caspases and nucleases; allowing selective cleavage and/or activation of select signalling pathways while protecting important cellular structures [92, 111].

Post-Translational modifications are analogous to Post-Translational modifcations affecting Apoptotic protein function. By altering the function of Enzymes/Protein Enzymatic Specificity, or When Protein/Protein Interactions occur (Phosphorylation, Ubiquitination, and, numerous other Redox modifications), Post-Translational modifications will create a bias toward Survival vs Death. In addition to changes in Inhibitor Stoichiometry for various forms of Inhibitory Proteins (IAP and ICAD), a buffering device that allows for Partial Activation without permanent commitment to Activation will be enhanced/manipulated [112].

From an evolutionary perspective, these mechanisms offer a competitive benefit by providing most cancer cells with the ability to capitalise on the same cellular stress-responsiveness pathways developed through the early evolution of cellular quality management. It's worth noting that DFFB is used as an illustrative example of rewiring the apoptosis process since its original purpose was to remove cells that had been irreparably disrupted; however, it now serves a new function of creating targeted genotoxic stress, which allows for tumour adaptation and evasion from detection by the immune system [113].

3. Type I Interferon Signaling in Cancer Immunity

3.1. Interferon Pathways: Canonical and Non-Canonical Activation

Interferons type I (IFN-I), particularly IFN-α and IFN-β, represent a major axis linking nucleic acid sensing to intrinsic cell growth control and the activation of immune responses. The canonical IFN-I pathway is activated via the recognition of pattern associated molecular patterns (PAMPs) associated with aberrant nucleic acids. The cGAS-STING pathway senses cytoplasmic double-stranded DNA (dsDNA), while RIG-I-like receptors detect cytoplasmic dsRNA and activate MAVS (Mitochondrial Anti-Viral Signaling) protein. Both pathways converge through activation of TBK1 (TANK-binding kinase 1) and IRF3 or IRF7 factors, resulting in the production of IFN-I and activation of the downstream JAK/STAT pathway via the Interferon Receptor (IFNAR). Eventually, this causes the induction of Interferon Stimulated Genes (ISG), which include genes with anti-proliferative, pro-apoptotic and immunostimulating activities [50, 114].

In addition to the canonical IFN pathways listed above, many tumors also respond through a non-canonical route activated by intrinsic (endogenous) immunostimulatory nucleic acids. Damage to DNA, chromatin instability and replication stress create conditions that generate micromini nuclei and cytosolically located DNA that activate the cGAS pathway independent of an infectious agent [115]. There is a second non-canonical route initiated by the activation of retroelements (episomal) where double-stranded RNA activates RIG-I and MDA5 and cascades into the IFN-I pathway [116]. The identification of these two additional non-canonical pathways provides a broader view of how IFN-I activation occurs resulting in the interpretation that IFN-I acts as a continual monitor of genomic stability, not just as an induced response to infection.

The biological response generated by IFN signaling is not strictly "yes or no", but rather determined by how much signal is received, how long the signal is received, and where the signal is received. High intensity and short duration of IFN signaling correlate with immunity-mediated tumor destruction; low intensity and prolonged duration of IFN+ signaling can promote the induction of adaptive responses resulting in immune tolerance, tumor growth stop (but survival), and/or lineage plasticity [117, 118]. Therefore, this context dependency explains the paradox of IFN I's ability to function as both immuno-suppressor and drive for immune evasion.

3.2. Interferon Surveillance and Tumor Cell Intrinsic Resistance

Tumor cells use IFN-I signaling as a method of internal monitoring that inhibits cell growth, enforces

cell-cycle checkpoints, and increases the presentation of tumor antigens. ISGs control the activity of p53, the fidelity of DNA repair, and the susceptibility of tumor cells to apoptosis, linking the immune system's ability to detect tumor cells to the control of their growth [119]. However, sustained oncogenic stress exerts strong selective pressure on tumor cells to downregulate the IFN-I signaling pathway, leading to the high prevalence of tumor-intrinsic IFN resistance.

Inhibition of the IFN signaling pathway occurs due to epigenetic silencing through DNA methylation or modification by repressive histones. To an extent, there are also a variety of other post-translational mechanisms involved in inhibiting IFN signalling [116]. Some of these include increased expression of SOCS proteins; IFNAR1 degradation and negative regulation of the IFN–JAK–STAT signalling pathway occur even in the presence of ligand binding [120]. Changes in cellular metabolism, such as dysfunctional mitochondria and altered redox states, also affect the responsiveness of cells to IFN. Changes in these metabolic pathways inhibit signaling via MAVS and activation of the IRFs [47].

A key point about IFN resistance is that it is often associated with specific lineages. Some transcriptional programs (e.g. MYC, RAS) are driven by oncogenes to actively suppress responsiveness to IFN. Additionally, hypoxic conditions create 'memories' within the cell that persistently suppress the ability to respond to IFN even after being returned to normoxia [121, 122]. These findings can be interpreted as indicating that IFN signalling constitutes an ongoing source of selective pressure that has influenced the entire process of tumour evolution, rather than a short-term response to immune stimuli.

3.3. Interferon Signaling in Cancer Stem Cells and Persister Cells

Recent studies have shown that tumor initiating cells are less likely to respond positively to interferon therapy than other (bulk) tumor cells, making interferon resistance a hallmark of MRD. Using a single-cell analysis approach, it was found that tumor cells surviving therapies contained a larger proportion of inactive, slow-cycling cells than did the bulk of tumor cells; these inactive cells had lower rates of basal interferon signaling, expressed fewer interferon stimulated genes and were less able to present antigens than the other tumor cells [123, 124].

This decrease in activity originates from the establishment of an active and chronic state of low level interferon signaling (through exposure to continual low doses of interferons or through repeated exposure to toxic levels of interferons), such that these cells do not respond to IFN treatment by being killed via the immune response, as would be expected [125]. Over time, such an environment creates conditions promoting the survival and growth of cell populations that can dissociate IFN signaling from its cytotoxic effects while still having some protective characteristics (e.g. the ability to tolerate damage to DNA) and/or prefer to be in a non-cytoxic state.

Two recent publications by Baldacci et al., and Selvaraj et al., demonstrating that persister cells exhibit not only transcriptional and epigenetic memories of previous exposure to IFN (IFN-c or interferon-c) but also, as such, have an ongoing, durable effect on their individual levels of responsiveness to IFN. In this respect, this represents an example of Adaptive Resistance wherein the ongoing immune pressures result in changes in plasticity being due to ongoing immune attack or pressure, which leads to the selection of persistently immune-evasive tumor cells. In addition, this presents evidence of a significant duality to the range of cytokine-based approaches now being utilized to treat patients with malignancies, as both supportive and inhibitory effects can now be attributed to specific types of IFN signaling through persistently immune-evasive tumor cells [126, 127].

3.4. Crosstalk Between Interferon Pathways and DNA Damage Response

Type I IFN signaling interacts closely with the DNA damage response (DDR) to create a circuit that connects immune surveillance with genomic instability through two-way communication. Examples of types of DNA damage that can be sensed by Type I IFN signaling include DNA double-strand breaks, mis-segregation of chromosomes, and replication stress, as well as the subsequent formation of cytosolic DNA in response to the activation of cGAS-STING by elevated levels of cytosolic DNA. Conversely, the activation of Type I IFN signaling will also influence or modify the cell's choice between distinct DNA repair pathways and the timing of cell cycle progression [115]. Thus, the interconnection between DDR pathways and Type I IFN responses enables both the sensing and reducing of genomic instability within the cell.

Unfortunately, there is often a "rewiring" of this DDR-IFN signaling network in cancerous cells. Genotoxic (therapeutic) stresses and/or oncogene-induced replication stress generate sub-lethally

damaging levels of DNA damage, resulting in activation of DDR networks without generating sufficient Type I IFN production to initiate Type I IFN signaling. Apoptotic proteins, notably caspases, nucleases, and dysfunctions in mitochondrial proteins due to mitochondrial membrane depolarization and NLRP3 inflammasome activation, can modulate the signalling pathways used by the cell in the context of DNA damage by determining how the cell accumulates and processes cytokine signals associated with cytosolic DNA damage [128].

Recent studies show that viable tumor cells actively inhibit immune recognition of tumor cells through controlled DNA fragmentation and repair of DNA damage response, thereby preventing immune detection but also paving the way for genetic adaptation [120]. This finding shifts from viewing the interaction between DNA damage response and IFN signaling as a cascading activation through a linear pathway to identifying and viewing this interaction as a dial or knob that has the potential to be fine-tuned by the cancerous cell through oncogenes. In this new light, the 'rewiring' of tumor cells associated with apoptosis has become a new mechanism to decouple the genotoxic effects associated with tumor cells from their activation by the immune system.

4. Cancer Persister Cells, Minimal Residual Disease, and Tumor Relapse

4.1. Defining Persister Cells: Origins and Properties

Transient therapy-tolerant cancer cells called "persister" cells are able to survive typically fatal treatments without having any prior mutations conferring resistance to those treatments. This type of cell forms the cellular foundation for the existence of MRD, which remains after treatment appears to have worked, and from which they give rise to recurrent cancer [129]. Rather than having a mutational basis for resistance, persister cells are viewed as being equally plausible to have a reversible phenotype due to being able to pause in an inactive state, and upon use or removal of therapy, return to being proliferative and, in some cases, regain drug sensitivity. This aspect of the definition of "cancer persister cells" is particularly important, as it casts treatment failures as being not only the direct result of the accumulation of mutations in the patient's cancer cells but also a reflection of the inherent adaptability of cancer cells in response to environmental stressors [130, 131].

n terms of functionality, persister cells exhibit a slow-cycling or quiescent phenotype, have the ability to withstand multiple forms of stress, and demonstrate a significant amount of metabolic remodelling. Evidence from multiple tumour types indicates that there is a trend towards less anabolic flux, alterations to mitochondrial function, and an increased reliance on alternate sources of power [132, 133]. The evidence suggests that persister cells are programmed to survive rather than proliferate, and that these characteristics are not fixed, but rather are dynamically induced in response to therapy. It supports this assertion by showing that when researchers analyse the chromatin landscape of persister cells using epigenomic analysis that it closely resembles that of other transformed cells, but distinctly contains reversible chromatin modifications that can revert back to a differentiated state once reactivated [134, 135].

4.2. Darwinian and Lamarckian Mechanisms of Persistence

The rise of persister cells is viewed as a combination of Darwinian selection and Lamarckian adaptation. In Darwin's theory, a treatment only favours rare pre-existing variants that already possess inner tolerance however many studies on multiple lineages and recordings on just one lineage have shown that this theory alone cannot explain the wide variety of persisters, or the repeatability of their phenotype. A second model, based off of Lamarck's theory of adaptation, has arisen through the idea that the experience of suffering a treatment can lead to a more adaptive phenotype through transition from an adaptive state to a non-adaptive state [70, 136].

Epigenetics plays a key role in the transition between adaptation and non-adaptation. Reprogramming of chromatin as a result of treatment, including changes to enhancer states and histone modifications causes a new form of transcriptional control where proliferation is inhibited while stress response and survival mechanisms become active [134, 137]. In addition to the above, there has been evidence to support the idea that metabolic adaptations also play a role in the persistent phenotype through increased oxidative stress buffering or changes to lipid utilisation resulting in stability under constant therapeutic pressures [133].

Adaptive persistence has been linked to the ability to adapt non-genetically through different mechanisms and to evolve genetically through Darwinian evolution. Indeed there is a time-limited period for adaptation through mutations generated when environmental conditions remain stable. Specifically, APOBEC (the most common cause of mutations), erroneous DNA repair, and replication stress have been linked with the creation of mutational diversity in continuous populations [138, 139]. Thus there goes

hand-in-hand the two mechanisms of adaptive persistence followed by Darwinian evolution.

4.3. Persister Cell Regeneration and Clonal Evolution

Re-current tumor does not occur just as cells that had been dormant before but instead arises from modification of established characteristics related to adaptation plus evolutionary bottlenecks. Studies looking at family trees of cell lines using both lineage search and single cell sequencing have discovered that amongst many persister cells only a small fraction re-enter the active growth cycle resulting in à return of the original's tumor but in addition to having an altered genetic make up [135, 140].

Further more this influences the number of adaptive mutations or epigenetic changes to occur after re-entering into the active growth cycle. Therefore, the presence of this limiting factor greatly increases the potential for increased genetic mutation rates resulting in additional altered gene structures and phenotypes [138, 141]. For this reason, there is a continued investment in the understanding of tumorogenesis among the bio-medical industry, and therefore, the transformation of an indolent cell type into a proliferative cell line will become of great interest in the future, as shown in Figure 3.

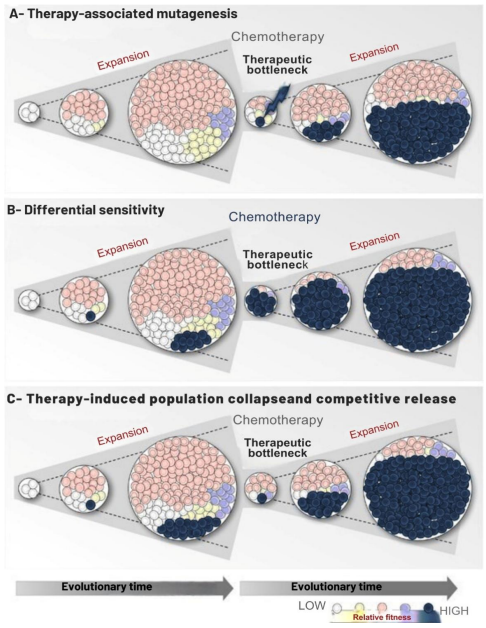

Figure 3: Conceptual models of therapy-driven tumor evolution following chemotherapy. A-Therapy mediated mutagenesis. B-Differential sensitivity. C-Therapy induced population relapse and competitive release.

Rather than supporting linear mechanisms of resistance evolution, our findings support that the processes leading to the generation of resistance work in a cycle, where therapy induces persistence, persistence provides a substrate for diversification, and diversification provides the fuel for relapse. This implies that persister cells do not simply serve as "survival" strategies for an individual cell in response to therapy; rather, they may serve as an active means to drive the evolution of the tumor.

4.4. Role of the Tumor Microenvironment in Persister Cell Maintenance

Although persister cell states are not inherently autonomous, tumor microenvironmental signals reinforce persister cell states. Metabolic gradients, immune infiltrates and tumor-associated stroma create niches which maintain persister cell states. Niche formation is promoted through therapy induced changes within the tumor microenvironment, including vascular injury, inflammatory signals and immune cell reprogramming [142, 143].

A key characteristic of these niches is immunosuppression. As several studies indicate, persister-enriched tumors have impaired interferon signaling, reduced levels of antigen presentation, and higher levels of immunosuppressive cytokines and checkpoint ligands [133]. As a result, this alteration of the tumor immune environment protects persister cells from being cleared by the immune system and limits immune-mediated elimination of persister cells during early growth. The inhibition of immune access through factors derived from the stroma and the composition of the extracellular matrix further contributes to the creation of niches which provide survival signals that support quiescent, stress tolerant cell states [144].

The phenomenon of metabolic crosstalk within the tumor microenvironment supports the maintenance of persister cell states. The presence of metabolic stressors such as hypoxia, nutrient deprivation and altered availability of biochemical metabolites favour the selection of tumour cells with flexible metabolic capability, linking persistence with environmental stress [137]. As an important point, these environmental characteristics continue to be present after completion of therapy, prolonging the duration of MRD and increasing the probability of relapse.

5. DFFB as a Molecular Regulator of Interferon Suppression and Persister Cell Regrowth

5.1. DFFB Activation in Sublethal Apoptotic Signaling

The protein called DFFB (DNA Fragmentation Factor Subunit B), which is also called CAD, has long

been viewed as the point of no return for cell death, or apoptosis, because it is normally activated after the cutting of its inhibitor, ICAD (Inhibitor of CAD), by caspase-3. However, new research demonstrates that DFFB can also be activated in sublethal conditions of apoptosis when caspase-3 is not able to perform its cellular function to create complete cell death but is still present [145].

The partial activation of DFFB under these conditions happens frequently in tumors receiving therapy, particularly in those with MRD as the residual cells are severely stressed through significant genotoxic stress and metabolic stress without undergoing complete apoptosis. Additionally, DFFB is able to function under low levels of procaspase-3 while avoiding the massive protein degradation that occurs during apoptosis [89, 146]. Moreover, DFFB serves more as an adaptive stress response than a trigger for nuclear collapse, further preventing the nuclear disintegration of cells displaying the molecular signatures of apoptosis, but not progressing to the point of establishing cell death as evidenced in persister cells that possess molecular markers of apoptotic action without losing viability. Furthermore, these findings allude to an emerging concept of non-lethal apoptotic signaling that can confer survival advantages during acute or chronic treatment with a therapeutic agent [147].

5.2. DFFB-Dependent DNA Damage and Mutagenesis

The sublethal activity of DFFB causes a unique form of controlled fragmentation of the genome through a series of DNA breaks that differ from the extensive cleavages that occur during apoptosis. The controlled damage to DNA is localized and consists of a multitude of lesions including the formation of double-strand breaks, fragmented regions of chromatin and the induction of a canonical pathway of DNA damage response (DDR) that executes repair pathways without causing loss of viability [147]. In other words, DFFB controlled damage to DNA will stimulate ATM/ATR signaling through the activation of checkpoints and subsequently allow repair of the damaged DNA, thereby permitting cells to undergo the high levels of genomic instability while remaining alive.

Notably, the damage to DNA caused by DFFB is not strictly a byproduct of cellular stress, but serves to promote an adaptive evolutionary change. The process of repairing the lesions caused by DFFB in an error-prone manner results in the production of mutations that provide new genetic variation for evolution through the selection of individuals that survive during the outgrowth of persister cells or during tumor relapse [148, 149]. While this evolutionary process is consistent with existing models of mutation following therapy, the mechanism by which DFFB creates this opportunity is fundamentally different in that this process is enzymatic through the action of the apoptotic nuclease and not due solely to the replication stress. Therefore, DFFB provides an additional mechanism for generating genomic plasticity, thereby enabling persister cells to explore various adaptive pathways while remaining viable.

The DDR is neuropathogenicly anti- and part of the molecular bridge to apoptotic dysfunction and adaptive evolution. These events are functional, or rather working toward the same end (for example, DFFBs create pathways to confer stress tolerance to cellular systems that promote a greater chance of survival). These repair pathways often create an equilibrium between cellular survival during repair and diversification of the cellular state through decreased fidelity associated with the lethal effects of cellular damage caused by apoptosis [98, 150].

5.3. DFFB–ATF3 Axis: Suppression of Interferon Response

The major effect that DFFB has, because of damaging DNA, is activating the transcriptional programmes that respond to stress to inhibit the pathway of type I interferon signalling. Recently, ATF3 was identified as a critical factor that connects the response of DNA damage caused by genotoxic stress and immunosuppression to ATF3, an AP-1 transcriptional factor that is induced by stress [79]. DFFB mediated DNA damage causes activation of both the DDR and the stress Kinase pathways that converge on ATF3 thereby causing global transcriptional repression of interferon-stimulated genes (ISGs).

ATF3 acts as a transcriptional brake on the type I interferon response by suppressing IRF- and STAT-mediated gene expression. The ability to do this occurs in the presence of and intact sensing machinery upstream to cytosolic DNA. This indicates that inflammatory (DFFB) signalling blocks the activation of interferons at the transcriptional level, rather than preventing their activation [1, 151]. This is an important mechanistic distinction since the persister cells do not block the sensing of immune signals; rather, they convert genotoxic stress into a dominant transcriptional suppressive signal that prevents growth via interferons.

The DFFB-ATF3 connection provides a different perspective in interpreting interferon suppression as an active adaptive response to stress rather than a passive inactivation of function. Furthermore, the DFFB-ATF3 connection provides a mechanistic explanation of how persistently generating tumours from continual DNA damage prevents being immunogenic, thus explaining the long-time paradox in cancer immunology.

5.4. Escape from IFN-Enforced Growth Arrest and Tumor Regrowth

Signaling through Type I Interferons creates a significant barrier to cellular growth via its ability to hardwire cells in a senescence-like state or hold them in an arrested cell cycle, while increasing their visibility to the immune system. When chemotherapy has been administered to tumors, the unused Interferon signaling (IFN) pathway will contribute to the prolonged dormancy of the tumor and assist in reducing the chances for immediate relapse following treatment. However, the ability of tumor persisters to transcend the IFN signal blockade is facilitated via Downstream FFN Bias (DFFB) induced pathways [152, 153].

DFFB pathway-induced suppression of Interferon Stimulated Genes (ISGs) and downstream Antiproliferative Programs allows the tumor persisters the ability to re-enter the cell cycle once the Therapeutic Pressure (TP) has been removed [95, 149]. However, the ability of a tumor persister to escape IFN-mediated arrest is progressive, with a gradual change in stress versus growth signals, whereby the tumor cell ultimately expands clonally. The increased proliferation of the tumor cells due to this ability to replicate following a decrease in therapy from having escaped arrest also reduces antigen presentation and the ability to be recognized by the immune system.

Therefore, the action of DFFBs bridges the gap between the ongoing sublethal apoptotic signaling of tumor cells and the two distinct characteristics of tumor recurrence: the resumption of proliferation and the decreased amount of immune control over the tumor [154, 155]. Thus, the role of DFFBs in cell proliferation, in conjunction with the role of the Interferons as a barrier to proliferation, suggests that while not necessarily being the sole driver, DFFBs assist in promoting the change in tumor cells from a persistent stage to a recurrent state.

5.5. Evidence from Experimental Models (in vitro, in vivo, single-cell genomics)

The evidence for this model comes from convergence across experimental paradigms. In vitro, therapy-treated cancer cells upregulate Dffb activity, poorly activate caspase-3, elevate ATF3 expression, and inhibit IFN signaling but do not further undergo apoptosis [156]. Genetic or pharmacologic loss of DFFB rescues ISG expression and sensitises cells to IFN-mediated growth suppression, supporting the functional importance of DFFB.

In vivo models support these results. Residual tumors following targeted therapy are enriched for DFFB-activated, IFN-depleted cells that are preferentially capable of initiating recurrent disease [79, 157]. Single-cell transcriptomic analysis has identified a conserved state, which is marked by elevated ATF3 expression and suppressed IFN programs and DNA damage tolerance, that is observed in multiple cancer types across different therapeutic settings [130, 158]. Significantly, these signaling states are reversible in nature and suggest a transient but powerful influence of DFFB-mediated signals on defining tumor fate.

6. Therapy-Induced Immune Evasion Mechanisms

6.1. Apoptotic Protein-Mediated Immune Suppression

While therapeutic regimens such as chemotherapy and targeted therapies commonly target the apoptotic machinery within tumor cells, the partial activation of caspases, nucleases, and mitochondrial apoptosis proteins leads specifically to the generation of a stress response as opposed to cell death, which leads to the attenuation of immune responses through the production of signals inhibiting immune detection [159]. The use of caspases to break down nuclear and cytosolic proteins leads specifically to the inhibition of the production of immune-stimulating danger-associated molecular patterns (DAMPs), together with the regulation of the secretion of pro-inflammatory cytokines in a manner specifically inhibiting the induction of the innate immune response [149]. Additionally, this sublethal cell response is taken through specific adaptive transcriptional pathways which inhibit antigen and ISG expression, placing the tumor cells in a state of dormancy as a result of therapeutic intervention.

6.2. Interferon Pathway Rewiring in Therapy-Resistant Cells

Resistance is always accompanied by the switching of type I interferon (IFN-I). This is because the cells under genotoxic stress or targeted therapeutic pressure selectively acquire a loss of sensitivity to the inhibitory effects of interferon. This is through

epigenetic loss-of-function of interferon pathways and an increase in the turnover of interferon AR1 through the ubiquitin-proteasome pathway and the enhancement of negative regulators like the SOCS proteins. This is followed by the maintenance of a stressed phenotype under interferon exposure. This leads to the selection of a population of persister cells [92, 160].

6.3. Role of DFFB in Immune Evasion and Tumor Relapse

DNA fragmentation factor subunit B (DFFB) has a crucial function in merging sublethal signals of apoptosis with immune evasion. Partly activated DFFB acts on localized DNA damage that initiates the response to DNA damage, rather than inducing apoptosis [161]. Crucially, this regulated activation leads to the induction of stress response transcription, especially via the ATF3/AP-1 complex, thereby down-regulating ISG expression and further suppresses the signaling of type-I IFNs [1, 162]. Significantly, by inhibiting IFN growth as well as functioning actively as an immune suppressor, the ability of persister cells to survive under therapy as well as returning to proliferative activity culminating by the relief of pressure from therapies can be realized by the predominance of cells differentiated by activation via DFFB, lacking IFNs, that comprise the MRD [163]. Thus, besides determining the function of inducing apoptosis, a regulated function for DFFB as a differentiation facilitator for immune evasion & regrowth can be attributed.

6.4. Interplay with Other Immune Checkpoints and Evasion Strategies

The induction of immunosuppression through therapy rarely stops at the silencing of the IFN pathway. DFFB-mediated signaling intersects with canonical immune checkpoints such as PD-L1, CTLA-4, and TIM-3, as well as metabolic adaptations that regulate T cell infiltration and function [164, 165]. Tumor cells in the DFFB-activated state express differential cytokine secretion profiles, which broadly foster local immunosuppression and stromal remodeling conducive to persister cell survival [149]. Altogether, this multi-layered shield against both innate and adaptive immune recognition results from the combination of apoptotic redirection, IFN suppression, and checkpoint modulation - an integrated network that impedes MRD from immunotherapeutic pressure. This integrated network is context-dependent, varying across tumor types, treatment modalities, and microenvironmental conditions, further reflecting the complexity of targeting immune evasion in disease relapse [166, 167].

7. Unresolved Questions and Controversies

7.1. DFFB Isoform Switching and Functional Heterogeneity

Today, while DFFB is known to play an important role in sublethal apoptosis signaling and persister cell adaptation, isoform heterogeneity has been imparting high levels of biological ambiguities. The transcriptomic profile reveals that cancer cells could express alternative isoforms of DffB with varied nuclease activity, localization, and ICAD activity [1, 100]. The biological role played by isoforms in DFFB in relation to persister cell development during therapy has not been very well characterized. There could be isoforms that could favor controlled DNA fragmentation processes over apoptosis signaling and vice-versa. Instead, there could be a regulated isoform shift based on prevailing stresses and microenvironments. The debate on this subject assumes importance since isoform heterogeneity could cause differing abilities among persister cells characterized by their tolerance against DNA damage, suppression in interferon signaling, and suppression in seed replication [97]. There is an important need for biological dissection at the isoform resolution with functional attributes, implying that the biological understanding in relation to DFFB has several important unmet needs.

7.2. Context-Dependent Roles of DFFB in Different Cancer Types

It would appear that the involvement of DFFB is extremely context-dependent, with evidence pertaining to both survival and apoptosis roles. Although Dfb-dependent inhibition of IFN signaling and enhancement of persister cell regrowth are demonstrated in solid cancers such as breast and colon cancers [157], hematological malignancy and epithelial cancers may present with different implications, including those that can modulate the ease of access, chromatin tolerance, or DNA damage [168, 169]. These mechanisms by which DFFB exerts these context-dependent roles are as yet unresolved. Thus, the question is how the activation of DFFB is controlled by tissue-specific transcription or the microenvironment, and whether the expression levels of its co-factors such as ICAD or repair molecules differentially influence the survival capacities of DFFB with regards to DNA breakage versus cell killing [170, 171].

7.3. ATF3-AP1-ISG Crosstalk: Mechanistic Ambiguities

DFFB-induced ATF3 expression and the consequent repression of ISGs is thus seen to represent the pivotal hub within persister cell adaptation, and there is much to clarify from the perspective of mechanisms utilized here. First, the DFFB-induced signal transduction from DNA fragmentation to the

induction of ATF3 remains incompletely understood. Although DDR kinases and stress-induced MAPK activation were proposed to play roles here, the role played by cytosolic DNA signaling and nuclear damage-induced signaling is also unclear at this point [79]. Second, ATF3 is part of the AP-1 transcription factor with diverse conformation types based upon the type of JUN and FOS subunits, together with chromatin and post-translational modifications. Whether the ATF3-containing AP-1 transcription factor contributes to the repression of ISGs and potential activation of select others within persister cells also remains unexplored at this point. Lastly, the relationship between this hub and other negative regulators of the IFN signaling response and the potential utilization of mechanisms to compensate and restore the adaptive response to persister cells remains entirely unexplored at this point. This remains largely relevant when considering the current efforts to utilize this hub to target MRD with therapies to restore IFN-induced adaptive immunity [172, 173].

7.4. Heterogeneity of Interferon Signaling in Persister Cell Populations

Persister cell populations also inherit heterogeneity with regard to IFN signaling, and the origin and implications of this heterogeneity remain unclear. Recent single cell analysis of persister cell transcriptional profiles indicates a population of persister cells that co-express low levels of ISG but high levels of DFFB and ATF3, indicating that other regulatory factors and processes, such as epigenetics and/or the microenvironment, play important roles in the regulation of the IFN response [174, 175]. Moreover, the specifics of IFN down-regulation and re-induction in response to intermittent treatment or modulation of the microenvironment remain unclear. The implications of the heterogeneity of persister cell IFN signaling, potentially influencing survival, immune evasion, and consequent clonal expansion, remain unexplored, and addressing this issue requires the use of single cell analysis with IFN receptor, DFFB, and ATF3 perturbations [176, 177].

8. Future Directions and Clinical Implications

8.1. Targeting DFFB and ATF3: Novel Therapeutic Strategies

The recent understanding of DFFB's role in modulating non-malignant apoptotic pathways and suppression by interferon presents both opportunities and challenges in targeting it. Direct inhibition of the nuclease activity of DFFB is a difficult goal because of the critical role of this enzyme in physiological apoptosis and the absence of a profound and druggable active site, although there could be some indirect approaches. These approaches include the following: (1) Stabilization of the natural inhibitor, ICAD (DFFA), through the use of small-molecular glue compounds; (2) Modulation of the activity of caspase-3 through sub-apoptotic concentrations of selective caspase-3 inhibitors; (3) Use of allosteric inhibitors that could interfere with the binding of DFFB to chromatin or any of its co-factors in the persister cell populations, thereby specifically reducing the role of DFFB in supporting cell survival [95, 178, 179]. There is, however, an emerging rationale for indirect targeting strategies focused on non-lethal functions selectively modulated by DffB. The very latest biological analysis now indicates that DffB persister function is inextricably coupled with caspase activity and chromatin accessibility alongside DNA damage response signaling, implying an increased viability in pathway modulation against DffB over direct targeting [177, 180]. The use of synthetic-lethal DFFB-DNA damage sensitivity in the ATN-CHK1/replication stress response pathway presents one such highly promising but largely preclinical option.

ATF3, which is downstream of the DFFB stress signaling response, also offers a similarly albeit distinctly divergent targetable platform. ATF3 is known to function both as a transcription factor during the stress response and pleiotropically across various biological pathways. Thus, the transcription factor would not likely serve as an immediately targetable molecule with minimal systemic side effects against the stress response. However, the transcriptional target pathways mediated by ATF3 could serve as more amenable platforms to intervene upon, including the AP-1-mediated repression of interferon stimulated genes' transcription [71, 95]. Modulators of the epigenome, transcription factor cofactor release agents, and inhibitors of the upstream stress kinases that preferentially reduce the activation of ATF3 during anti-stress response efforts against tumor cells could also represent pathways to resensitize the resilient members to the immune response challenge.

The following strategies could be explored for the selective inhibition of the pro-survival activity of DFFB in persister cells without touching its essential role in physiological apoptosis. First, small-molecule "molecular glues" may be utilized to pharmacologically stabilize the DFFB-ICAD (DFFA) complex and, by this means, protect from caspase-3-mediated release and activation [181]. In addition, low-dose or locally delivered caspase-3 inhibitors may be considered to attenuate sublethal DFFB activation without ablating full apoptotic execution [182, 183]. Next, allosteric inhibitors, identified through fragment-based screening against noncatalytic sites, disrupt DFFB's chromatin binding or oligomerization

specifically in stressed cells [184]. Finally, cell-permeable peptide mimetics based on ICAD's inhibitory domain, or advanced modalities such as proteolysis-targeting chimeras (PROTACs) designed to degrade the activated form of DFFB, are highly specific and potentially tunable interventions [185-187]. All these approaches are at risk of clinical translation due to therapeutic index concerns; thus, transient dosing schedules will probably be needed together with tumor-localized delivery systems and combination with immunotherapies or genotoxic agents [188].

Thus, DFFB targeting needs to be done very intelligently, considering selectivity and safety. Complete inhibition of DFFB will disturb the normal apoptotic clearance and probably result in autoimmune or inflammatory pathologies. To avoid this, one might also consider conditional knockout or degraded systems in future, such as PROTACs, which would only act within tumor micro-environments. Combination regimens in which DFFB inhibition is combined with pro-apoptotic agents, such as BH3 mimetics, to selectively lower the threshold toward apoptosis in cancer cells. Biomarker-driven patient selection based on, for example, high expression of DFFB/ATF3 in MRD, to enrich for a population most likely to benefit from these strategies. These approaches are considered with the intention of maximizing the therapeutic window to avoid toxicity linked with broad disruption of apoptosis.

Cancer therapy results in the initiation of sublethal signaling for apoptosis, along with partial activation of caspase-3, which leads to DNA damage via DFFB without any decrease in cell viability. This signaling down-regulates the innate interferon pathways, as well as antigen presentation, so as to facilitate immune evasion, thereby ensuring the survival of persister cells, as shown in Figure 4.

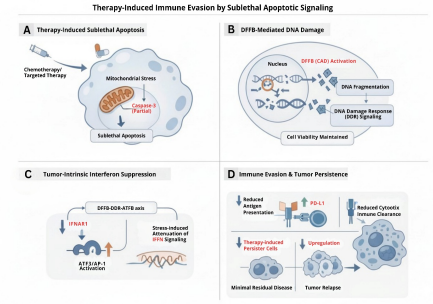

Figure 4: Therapy-induced sublethal apoptosis promotes immune evasion and tumor persistence. A-Therapy-induced sublethal apoptosis, B-DFFB-mediated DNA damage, C-Tumor- intrinsic interferon suppression, and D-Immune evasion and tumor persistence.

8.2. Combination Therapies: DFFB/ATF3 Inhibitors with Immunotherapy

The most compelling clinical rationale for targeting the DFFB-ATF3 axis lies in combination strategies with immunotherapy. Persister cells, characterized by reduced interferon signaling and impaired antigen presentation, are intrinsically resistant to immune checkpoint blockade, despite otherwise favorable tumor mutational profiles [100, 189]. Preclinical evidence suggests that restoration of interferon capacity, even transiently, can reactivate immune-mediated elimination of residual disease. In this context, pharmacological or genetic disruption of DFFB-ATF3 signaling can be used to permanently expose cells to immune attack rather than as a stand-alone cytotoxic strategy.

Checkpoint inhibitors targeting PD-1/PD-L1 or CTLA-4 may be particularly effective when combined with approaches that reverse DFFB-driven interferon suppression, as interferon signaling directly regulates both antigen presentation and checkpoint ligand expression [149]. In addition to T cell-centric therapies, NK cell-based approaches are also of interest, given their sensitivity to interferon-regulated stress ligands and their ability to eliminate slow-cycling, antigen-less tumor cells [141, 157]. While direct clinical evidence is lacking, these combination strategies are mechanistically based and consistent with broader efforts to overcome therapy-induced immune evasion by targeting tumor-intrinsic stress adaptations rather than immune checkpoints alone.

8.3. Biomarker Development for Persister Cell Detection

Translating DFFB and ATF3-centric concepts to the clinic will require robust biomarkers capable of consistently identifying cell status and MRD. Bulk interferon signatures have already shown prognostic value in several cancer types, but their interpretation is confounded by immune cell infiltration and stromal contributions. More sophisticated approaches that focus on tumor-intrinsic transcriptional relationships, including repression of ISG expression combined with stress-response gene activation, may better capture persister biology [1, 148]. DFFB isoform expression or post-translational modification patterns represent another potential biomarker class, although technical challenges in detecting these functions in patient samples remain significant.

Single cell and spatial profiling platforms also provide hope because they enable direct detection of rare persistent populations within heterogeneous tumors. Alternatively, “marks” of DNA damage, like fragmentation patterns, could provide functional

assays of Dfb function that can be incorporated into circulating tumor DNA analysis to provide longitudinal analysis of MRD [149]. Although these new platforms are still emerging, they make a clear point that it is necessary to look beyond genomic biomarkers to new dynamic metrics of cellular state and adaptation to stress.

8.4. Clinical Trials and Translational Challenges

Although compelling mechanistic rationale supports the therapeutic promise of targeting DFFB or ATF3, several issues exist that can impede its success in the clinic. Chiefly, the difficulty of finding safe and approved inhibitors that specifically target the proteins is high. This is because the initial phases of the drug evaluation process will have to adopt already-approved drugs that target the pathways in an indirect manner. Another factor is the apoptotic and interferon pathways that are also responsible for maintaining the integrity of normal host tissue homeostasis [149, 190, 191].

Also, the problem of selecting patients remains unsettled. The fact that persister cell states are transient, treatment-driven, and, by all appearances, optimal opportunities are relatively short, means that modeling these phenomena may demand adaptive studies involving on-treatment biopsy, fluid biomarkers, and immune measures early in the course of treatment. It is important to note that any treatment success in these patients will be independent of the primary endpoint of tumor regression, but rather of relapse response, which will demand special endpoints based on persistence dynamics rather than conventional response [175, 192-200].

Together, these few future directions establish DFFB-mediated interferon suppression as a conceptual and translational bridge between non-canonical apoptosis, immune evasion, and tumor relapse. While direct therapeutic targeting remains difficult, indirect modulation of the DFFB-ATF3 axis, particularly in combination with immunotherapy, provides a mechanistically based approach for exposing resistant cells to immune eradication. Success will require sensitive biomarkers, innovative trial designs, and a willingness to move beyond transient, therapy-induced cellular states, which have traditionally fallen below the radar of both detection and intervention [95, 100].

9. Challenges and future prospective

Despite these advances, much remains to be understood and addressed. The isoform functional diversity of DFFB, the roles of DFFB and ATF3 in particular cancer types and environments, as well as the ATF3-AP1 complex-specific mechanism needs to be better addressed. In addition, as persister cells exist only temporarily, making direct targeting unfeasible, research initiatives should therefore aim at indirect targeting methods, such as developing on synthetic lethal DFFB-dependent DNA damage tolerance and altering upstream stress-activated kinases affecting the ATF3-mediated pathway of immunosuppressive function. The best way forward from here, therefore, appears to be developing combination therapies comprising interferon pathway-targeting and antigen presentation in persister cells combined with immunotherapeutic approaches that might help target persister cells' innate resistance to immune checkpoint blockade. "In order to successfully interpret these concepts, it is necessary that we develop 'dynamic biomarkers that can monitor persister cell states' within patient samples, as well as new clinical trials that take into account 'the biology of MRD.' Only by combining this understanding of adaptive persistence at a mechanistic level with innovation at the translational level can we offer a clear plan that will allow us to 'avoid relapsing and improve long-term outcomes' in cancer patients."

10. Conclusion

The review offers an entirely new conceptual framework in understanding MRD and therapeutic relapse. The overwhelming evidence supports that key components in apoptosis were originally conceptualized in dual roles in cell death as adaptors in cancer cell survival strategies against therapeutic selective pressures. The caspase-activated nuclease, DFFB, is pivotal in this new conceptual framework. Its activation in persister cancer cells initiates a controlled DNA damage response that even promotes genetic variation and initiates an immediate transcriptional program characterized by the transcription factor, ATF3, that actively represses the transcriptional activity associated with the signaling from the primary type I interferon DFFB. Thus, the DFFB-ATF3 axis becomes a pivotal molecular link between sublethal apoptosis, therapy resistance, and immune evasion. Based on these, we deduce that resistance is not merely a consequence of Darwinian selection of pre-existing mutations but is significantly influenced by Lamarckian, therapy-induced adaptive cell states. Persister cells constitute a malleable, ephemeral population whose endurance is augmented by atypical apoptotic signaling and microenvironmental factors, notably immunosuppressive niches. The rewiring of these cells' interferon pathways shows that IFN signaling is context dependent. It can act as a barrier to tumors and, when chronically weakened, as a factor that allows them to persist.

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Using Artificial Intelligence (AI): we used Quillpot for language rewrite

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Figure ligands

Figure 1: Type I interferon signaling regulates tumor surveillance, cellular plasticity, and therapy resistance through tumor-intrinsic mechanisms. A- Type I interferon signaling in tumor surveillance, cellular plasticity. B- Tumor-intrinsic mechanisms in cancer stem cells and resistance. C- Tumor-intrinsic mechanisms in cancer cells and persistence.

Figure 2: The role of the tumor microenvironment in driving epithelial–mesenchymal transition (EMT) and cancer cell plasticity.

Figure 3: Conceptual models of therapy-driven tumor evolution following chemotherapy. A-Therapy mediated mutagenesis. B-Differential sensitivity. C-Therapy induced population relapse and competitive release.

Figure 4: Therapy-induced sublethal apoptosis promotes immune evasion and tumor persistence. A-Therapy-induced sublethal apoptosis, B-DFFB-mediated DNA damage, C-Tumor- intrinsic interferon suppression, and D-Immune evasion and tumor persistence.