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Chemical Inductors of Proximity Associating DNA Damage-Binding Protein 2 and XIAP as Targeted Therapy for General Cancers

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Abstract

While all cancers are classified as uncontrolled proliferations of cells, they manifest in many forms and may arise from a wide range of cellular anomalies. It has thus made finding a universal targeted cancer therapy a highly desirable goal. Several common biochemical changes associated with oncogenesis have been described in literature across all cancers, namely the Warburg effect (Heiden et al., 2009) (Warburg, 1925) and resistance to apoptosis (Hanahan & Weinburg, 2011). These hallmarks possess high potential to be used as targeting mechanisms in cancer treatment. Recent findings by Gourlsankar et al. have shown a promising new technique of linking the activity of a frequently activated cancer driver (B cell lymphoma 6) with an apoptosis promotor, allowing the creation of a synthetic biochemical pathway which rewires cancer driver activity to induce cell death, done through using a drug to covalently link two molecules that bond to each protein (Gourlsankar et al., 2023). While this approach specifically targeted diffuse large B cell lymphoma, it shows high promise for new strategies to approach eliminating cancer cells, namely in the artificial activation of tumor suppressor function through protein linkage and the consequent association in protein location within the cytosol. This paper aims to review the strategy of Gourlsankar et al., identifying ways to expand its concept towards generalized treatments using the hallmarks of cancer, and proposing a new theoretical drug mechanism using the linkage of DNA damage-binding protein 2 (DDB2) with X-linked inhibitor of apoptosis (XIAP) to induce cell death.

Keywords: cancer, targeted cancer therapy, DNA damage-binding protein 2, X-linked inhibitor of apoptosis (XIAP)

1. The “Crabtree Method” — Linking Cancer Drivers and Gene Promotors

The method outlined by Gourlsankar et al., which we will refer to as the “Crabtree Method”, details the use of a transcriptional chemical inducer of proximity (TCIP) molecule, which is able to non-covalently link two molecules, with chemical moieties at the ends binding to a specific protein each, using an alkane chain to space apart the two proteins. This effectively creates a hybrid, where the activity of one protein, such as a constitutively-active cancer driver, influences the localization and transcriptional activity of the second protein (Gourlsankar et al., 2023). Using diffuse large B-cell lymphoma cell lines, Gourlsankar et al. is able to tether the transcriptional protein bromodomain-containing protein 4 (BRD4) to BCL6, which is commonly expressed in this cancer. BCL6 acts as a suppressor of pro-apoptotic, cell cycle arrest, and DNA-damage response genes through binding to DNA sites at transcription start sites. However, its location in close proximity to such genes allows BRD4 to, once tethered using the TCIP, induce transcription, and override the BCL6 blockage by recruitment of RNA polymerase II, which inherently interacts with the protein’s C-terminal (Itzen et al., 2014). Thus, activation of genes leading to proliferation arrest and cell death occurs by using BCL6 to anchor BRD4, which recruits RNA polymerase as a transactivation domain, as shown in Figure 1.

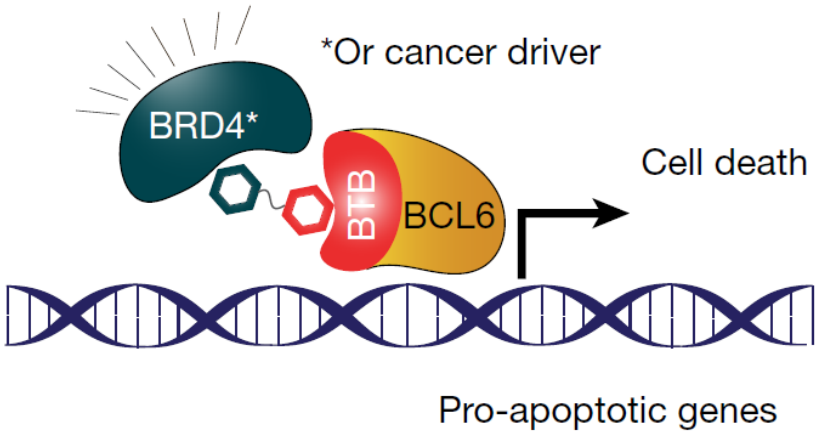

Figure 1. BRD4 and BCL6 tethered with the TCIP, with BRD4 recruiting RNA polymerase to transcribe pro-apoptotic genes (Gourlsankar et al., 2023)

The association of these proteins to achieve this effect is achieved through utilizing the molecules BI3812, which binds to the BTB domain of BCL6, and JQ1, which binds to the bromodomain of BRD4. By connecting BI3812 and JQ1 with an alkane chain, Gourlsankar et al. created a TCIP to link the two proteins molecularly.

Gourlsankar et al. tested the TCIP molecule with four distinct cell lines of diffuse large B-cell lymphoma, each with high levels of BCL6. This allows the Crabtree Method to work with high efficacy due to the high levels of BCL6 in the cell and the readily available anchoring to DNA. This molecule, named TCIP1, performed with the highest efficacy and lowest EC50 compared to the two negative controls, showing a marked effect through its activity. TCIP1 is able to kill the KARPAS422 cell line with a half-maximal EC50 only 72 hours after drug administration (Gourlsankar et al., 2023). Thus, one is able to conclude that the Crabtree Method is able to effectively tether two proteins through induced proximity and the TCIP molecule, associating their locations and effects.

1.1 Method Limitations

While the Crabtree Method has remarkable results when used for B cell lymphoma, it does not show similar levels of success when tested in generalized cancer use. Gourlsankar et al. tested TCIP1 with 906 cancer cell lines, selected randomly, with varying lineage and type. TCIP1 was found to be of greatest efficacy when tested with similar cancers to diffuse large B-cell lymphoma, such as in hematopoietic malignancies and lymphomas, where the anchor BCL6 was highly expressed (Gourlsankar et al., 2023). Thus, the method tested is dependent on BCL6 presence and its location in front of pro-apoptotic genes to kill cells. If BCL6 is not present in its desired location or is not highly expressed, then BRD4 is unable to perform its desired role of recruiting RNA polymerase for pro-apoptotic gene transcription and will instead cause transcription elsewhere.

In the pursuit of an all-encompassing but directed approach for treating cancer, the Crabtree Method is lacking in generality as it has been shown to be cancer-type specific. However, it has shown that chemically induced proximity of proteins has potential to be a treatment for cancer, either using cancer drivers, transcription factors, or regular cell proteins. In the search for a generalized treatment, one promising identifier present in many cancers is elevated reactive oxygen species (ROS) levels in the cytoplasm.

2. The Warburg Effect and Reactive Oxygen Species

The aerobic metabolism of normal cells consists of three steps: glycolysis in the cytosol, followed by mitochondrial Krebs cycle and oxidative phosphorylation through the electron transport chain and ATP synthase. This is the most effective energy pathway; it nets 36 ATP per glucose in its entirety, almost fully due to oxidative phosphorylation. However, when oxygen is lacking, the Krebs cycle is unable to proceed, and instead the pyruvate used is fermented to lactate. This means that glycolysis by itself is the sole metabolic process, which nets 2 ATP per glucose and is greatly less nutrient-efficient than mitochondrial metabolism.

Otto Warburg published in 1925 his discovery of the “Warburg Effect”, which states that cancer cells will prefer glycolysis and lactate fermentation rather than the energy-efficient oxidative phosphorylation, even in aerobic conditions (Warburg, 1925). This switch may be due to several reasons, such as the nature of requirements in proliferating mammalian cells, where they require a constant supply of glucose and nutrition to build biomass. Oxidative phosphorylation is seen as a nutrient-efficient process as it does not require much glucose, but in

cancer cells which are constantly provided with glucose, there is no incentive to stop using glycolysis, as the cell does not lack the proper growth signals and nutrition (Heiden et al., 2009). Furthermore, if glucose is in excess, glycolysis by itself can produce ATP in a much higher rate than complete oxidative phosphorylation (Liberti & Locasale, 2016), suitable for rapidly proliferating cells such as cancer. In support of this idea, it has also been found that embryonic stem cells, who have similar requirements to cancer cells in proliferation, have also prioritized the switch to glycolysis (Kondoh et al., 2007). Furthermore, the aneuploid nature of many cancer cells may also lead to these metabolic changes.

The switch away from mitochondrial function directly leads to the creation of excess reactive oxygen species (ROS), a hallmark of many cancers. As mitochondria produce up to 90% of cellular ROS (Tirichen et al., 2021), the cessation of mitochondrial activity leads to significant increases in superoxide ($O_2^{\bullet-}$) production (Murphy, 2009). Dysregulation of cellular ROS levels causes genomic instability, damaging DNA, proteins, and organelles. In regular cells, abnormally high amounts of ROS and DNA damage causes oncogenesis, but as ROS keeps building up from further genomic damage, the cell may be pushed to undergo senescence and apoptosis (Milković et al., 2019). However, cancer cells do not possess such functional tumor suppressors, such as P53, and instead continue living with elevated levels of ROS. Thus, ROS has potential to be used as an identifier of cancer cells against body cells, as any healthy cells are unable to sustain such elevated levels of ROS.

3. DNA Damage-Binding Protein 2 and ROS Detection

DNA damage-binding protein 2 (DDB2) plays a main role in DNA nucleotide excision repair (NER). It can activate and upregulate expression in response to ultraviolet radiation-induced damage, migrating to the nucleus and binding to the DNA to initiate NER (Stoyanova et al., 2009). Its normal function prevents tumorigenesis through this repair mechanism, typically of epithelial cells which are most affected by radiation.

However, DDB2 has shown to have a close relationship with ROS. Moreover, it has been identified that a positive feedback loop exists between the two, where ROS accumulation is able to induce DDB2 expression, such as that in cancer (Roy & Raychaudhuri, 2013). ROS accumulation is then promoted through DDB2-induced suppression of antioxidant genes, leading to a cascade of mutual activation. Similarly, cells lacking DDB2 do not increase in ROS following DNA damage (Roy & Raychaudhuri, 2013). Cancer cells have elevated levels of ROS through the Warburg Effect, with such species reacting and damaging DNA. The genomic instability, along with elevated ROS levels, makes DDB2 a likely candidate for detecting oncogenesis as it can react to ROS and bind to ROS-induced DNA damage.

4. Novel Therapeutic Approach — Linking DDB2 and Apoptosis Regulators Through Crabtree Method

The Crabtree Method shows how two proteins can be tethered to each other, associating the movement and position of one protein with that of another using a TCIP or plain CIP (chemical inducer of proximity. As DDB2 has a known tendency to move into the nucleus and towards DNA when ROS or genomic damage is detected, this migration can be exploited through connecting it with another protein which causes apoptosis. Thus, a targeted but generalized therapy can be achieved in cells with DDB2 activity. This paper proposes using the Crabtree Method and a novel CIP to associate the proteins DDB2 and X-linked inhibitor of apoptosis (XIAP), a regulator of apoptosis whose primary function of inhibiting caspase 3, 7, and 9 requires it to be present in the cytosol (Abbas & Larisch, 2020). If linked to DDB2 and brought into the nucleus, where there are relatively few caspases, XIAP is unable to perform its role of inhibition, thus allowing caspases to begin and carry out the apoptosis cascade.

4.1 Role of XIAP in Apoptosis Regulation

Apoptosis is a programmed cell death pathway, caused by intrinsic or extrinsic signals. It may be initiated due to excessive cell stress, genomic instability, or immune cell cytokine activity. Apoptosis involves the release of cytochrome c from mitochondrial pores, caspase protein degradation, and cytoskeleton disassembly, no matter the pathway (Uren et al., 2017). This results in complete cell death and fragmentation of important cell structures, eliminating cells deemed unfit to continue growth. In cancer, apoptosis is often in insufficient amounts due to upregulation of inhibition signals or growth factors, leading to proliferation.

XIAP is an inhibitor of apoptosis (IAP), the most potent member of this family of proteins, as it directly inhibits caspase function and thus stopping apoptosis. It contains three BIR inhibitor domains, binding to caspases 3, 7, and 9. Within the apoptosis pathway, caspases 3 and 7 cleave and disassemble proteins and cellular machinery, as well as cause cell detachment, whereas caspase 9 is an effector, recruiting other caspases. Thus, the inhibition of these caspases directly results in apoptosis disruption, supported by the frequent overexpression of XIAP in cancer (Abbas & Larisch, 2020).

The logic in moving XIAP into the nucleus through DDB2 association lies within the localization of caspases and their function. It has been found that nearly every caspase is predominantly present in the cytosol of cells, with minimal fractions present in the mitochondria and even less in the nucleus (Zhitovsky et al., 1999). Thus,

XIAP is significantly less potent in inhibition if brought into low concentration areas such as the nucleus. Moreover, activated caspases are almost entirely localized in the cytosol (Luo et al., 2010), where it is best able to cleave other proteins and recruit new caspases, and caspase activation occurs most frequently in the cytosol under effect of mitochondria-released cytochrome c and dATP, aided by the Apaf-1 apoptosome (Zhitovsky et al., 1999). Thus, even if XIAP was able to inhibit targets within the nuclear fraction, its effect would be low enough to potentially allow apoptosis to proceed.

4.2 Alternative Targets for DDB2 Association — Bcl-2 Family

Proteins of the Bcl-2 family are anti-apoptotic mitochondrial membrane members. During normal cell function, a homeostasis between Bcl-2 inhibitors and Bax family promotors of apoptosis must be reached to control mitochondrial outer membrane permeabilization (MOMP) and prevent cytochrome c release when unnecessary, as cytochrome c will initiate the apoptotic cascade through caspase recruitment (Bender & Martinou, 2013). Without sufficient concentrations of Bcl-2 to regulate the membrane, pro-apoptotic factors such as the Bax and Bak proteins will coagulate to form pores in the mitochondrial membrane and release cytochrome c, initiating apoptosis. Thus, Bcl-2 family proteins can be intercepted through binding to a CIP molecule in the cytoplasm, before association into the mitochondrial membrane, and taken away into the nucleus by DDB2 activation. If this happens in sufficient quantities, then the cytosolic concentration of Bcl-2 will be greatly reduced, and the mitochondrial membrane will have less Bcl-2 components. This then allows the imbalance in homeostasis to initiate the release of cytochrome c and apoptosis to follow.

One such target may be the induced myeloid leukemia cell differentiation protein MCL-1, a member of the Bcl-2 family. It has been found that its overexpression in cancer cells lead to antiapoptotic effects, and that its loss quickly leads to apoptosis (Senichkin et al., 2020). MCL-1's biological half life lasts less than an hour, leading to high sensitivity to changes in cellular levels (Senichkin et al., 2020). It contains three isoforms, with two being proapoptotic (MCL-1S, MCL-1ES) and one antiapoptotic (MCL-1L). MCL-1S can suppress the antiapoptotic effect of MCL-1L through dimerization, leading to a homeostatic-dependent balance for normal cell function (Wang et al., 2021). As cancer cells often break this homeostasis through overexpression of the antiapoptotic isoform, association with DDB2 and moving excess MCL-1L into the nucleus may reestablish the balance required for proper apoptotic signaling.

4.3 Caveats of Proposal

While this approach is not cancer-type specific and is more generalized than the initial Gourlsankar et al. trials, it suffers from the common pitfalls of cancer drugs, as it is highly susceptible to mutation within the genome and proteins. The dependence on proper DDB2 function and the nuclear migration means that mutations within DDB2, causing loss-of-function or erratic behavior, will nullify the treatment. If XIAP is not brought into a low concentration area such as the nucleus and is allowed to remain in the cytosol, then there is no lack of inhibitive targets for itself, and the treatment would have had no effect. Furthermore, when connected to a CIP and another protein, if DDB2 does not perform identically to the unbonded type, the nuclear migration will also not occur, failing the treatment. Moreover, if the cancer cell downregulates DDB2 or upregulates XIAP, then there may be too great a concentration difference for the treatment to effectively create a difference through removal of XIAP. The apoptosis pathway may also prove difficult to predict, as cancer cells notoriously are able to change biochemical pathway compositions and upregulate new inhibitors. As it is proven that apoptosis can occur independent of caspase function (Negara et al., 2020), the pathway may be altered so that caspase activation is not induced. It may also occur that caspases are mutated beyond functionality, losing their ability to initiate the cascade of apoptotic activities, and thus making XIAP removal from the cytosol useless. Lastly, this approach is fully dependent on finding molecules able to non-covalently attach to both DDB2 and the anti-apoptotic protein without disrupting their normal function. It is necessary that DDB2 properly anchors to DNA damage sites, and that XIAP still can inhibit caspases. Without this, the treatment cannot function in ROS-present cells and/or causes apoptosis in normal cells. To find molecules which can bind to these proteins without inhibiting their function, it is possible to screen various compounds through cell cultures to see which can bind and still have normal cellular function. Furthermore, it is also possible to computationally predict compounds that bind to specific locations where it would not interfere with normal protein function.

5. Conclusion

The proposed novel approach lies heavily upon theory and is ultimately untested. It demonstrates, however, the potential that Gourlsankar et al. have given through their use of CIPs and TCIPs, as well as the design of a wide spectrum, but targeted, cancer treatment.

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