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PERSPECTIVE

Cancer therapeutics based on BCL-2 functional conversion

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The BCL-2 protein family plays a key role in the regulation of 'intrinsic' apoptosis by regulating the integrity of the mitochondrial outer membrane (MOM). The BCL-2 family of proteins consists of both anti-apoptotic and pro-apoptotic members. These proteins possess one to four BCL-2 Homology (BH) domains and fall into three functional groups. The anti-apoptotic proteins include BCL-2, BCL-X_L and MCL-1, which possess BH1-4 domains. The pro-apoptotic BCL-2 family members are divided into two subclasses, comprising the BH3 only proteins such as NOXA and PUMA and multi-domain proteins such as BAK and BAX which possess the BH1-3 domains. The balance between the expression of pro- and anti- death BCL-2 proteins determines the cell fate as pro-survival BCL-2 members bind and inhibit the activity of pro-death members. The BH3-only proteins are upregulated upon cellular stress such as DNA damage or oncogene activation and then bind pro-survival proteins, preventing their inhibition of BAX and BAK. Furthermore, certain BH3-only proteins, particularly BIM and BID can directly activate BAX and BAK resulting in MOM permeabilization. MOM permeabilization triggers the caspase cascade which cleave vital cell proteins resulting in cell demolition. The BH3 domain is the critical death domain within the BCL-2 pro-apoptotic family members, which not only inhibits pro-survival proteins but activates pro-death proteins.

Cancer cells often exhibit increased expression of pro-apoptotic proteins resulting from DNA replication stress and cell cycle checkpoint evasion. However, cancer cells are able to survive by blocking these death signals. BCL-2 promotes tumorigenesis by enabling cells that would normally undergo programmed cell death to survive. Elevated expression of anti-apoptotic proteins such as BCL-2 can prevent apoptosis induction through sequestering pro-apoptotic proteins BAK/BAX and preventing their activation. Therefore, elevated expression of BCL-2 contributes to poor prognosis of Triple Negative Breast Cancer patients and confers resistance to chemotherapeutics (Honma et al. 2015). BCL-2 is also highly expressed in cancers that are refractory to therapy such as Non-Small Cell Lung Cancer. Early strategies of targeting BCL-2 focussed on antisense oligonucleotides to suppress BCL-2 expression. However, this approach has not progressed to successful clinical trials. BH3 mimetics such as ABT-737 that bind and neutralize the BH3-binding grooves on anti-apoptotic BCL-2 family members displace BH3-only proteins resulting in BAX and BAK activation. Recently, ABT-199 that inhibits BCL-2 has been approved for patients with chronic lymphocytic leukemia (CLL) as well as for small lymphocytic lymphoma who have received at least one prior therapy. However, it is likely that only cancers that are dependent on BCL-2 for survival will respond, which limits its utility in solid tumors. As tumors progress they shift their dependence from one survival protein to another within the BCL-2 family resulting in BH3 mimetic resistance.

An alternate strategy of targeting BCL-2 is through BCL-2 functional conversion. BCL-2 is converted from a pro-survival protein to a pro-death protein by binding to NUR77, a nuclear receptor that migrates from the nucleus to the mitochondria to initiate apoptosis. NUR77 interacts with BCL-2 within the unstructured loop region between the BH3 and BH4 domains. This interaction induces a conformational change exposing the hidden 'killer' BH3 domain. Exposing the BCL-2 BH3 domain releases BAX which leads to MOM permeabilization and the release of cytochrome c. In addition, the exposed BCL-2 BH3 domain inhibits other anti-apoptotic BCL-2 family members such as BCL-X_L. Therefore, this strategy not only neutralizes BCL-2's survival function, it directly activates pro-apoptotic proteins and

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further inhibits other pro-survival members. Thus, NUR77 converts BCL-2 from a protector to a killer of cancer cells.

NuBCP-9 is a nine amino acid peptide that mimics the mechanistic and functional activities of NUR77 and induces BCL-2 selective apoptosis in cancer cells (Kolluri et al. 2008). NuBCP-9 is effective against breast and lung cancers that express BCL-2. NuBCP-9 also suppresses paclitaxel resistant lung and breast cancer growth (Gupta et al. 2018; Pearce et al. 2018). NuBCP-9 effectively induces apoptosis in cancer cells that express BCL-2 phosphorylated at Serine 70, which confers resistance to various death signals (Pearce et al. 2018). However, in vivo delivery of NuBCP-9 has been a challenge. Encapsulation of NuBCP-9 into polyethylene glycol-polypropylene glycol-polyethylene glycol (PLA-PEG) nanoparticles has improved in vivo efficacy resulting in prolonged tumor regression (Kumar et al. 2014). Furthermore, NuBCP-9 and paclitaxel have been successfully loaded on PLA-PEG nanoparticles and this dual loading resulted in synergism and improved in vivo efficacy (Gupta et al. 2018). This work highlights the feasibility of nanoparticle delivery of NuBCP-9. New delivery methods, which enable temporal and spatial release have been reported utilizing hollow gold nanoshells and near infrared irradiation release. The potential to deliver and release NuBCP-9 in localized tumors is an exciting prospect for the treatment of solid tumors.

The strategy of targeting BCL-2 for functional conversion is effective in cancers that are not solely dependent on BCL-2 for survival. In contrast to BH3 mimetics that require cancer cells to be BCL-2 dependent, functional conversion relies on the presence of BCL-2 for induction of apoptosis. In addition, the effect of NuBCP-9 is potentiated with increase in BCL-2 expression, which is a common therapeutic resistance mechanism providing a rationale for its effectiveness against paclitaxel-resistant cancer as well as its synergy with paclitaxel. Thus, BCL-2 functional conversion is an effective strategy to treat chemoresistance and when used in combination with chemotherapy will likely reduce acquired resistance (Pearce et al. 2018). Upregulation of survival proteins such as BCL-X_L is a known mechanism of resistance to ABT-199. As BCL-2 functional conversion activates BAX/BAK and also inhibits BCL-X_L, this will likely prevent resistance mechanisms linked to upregulation of survival proteins. The tolerability and lack of systemic toxicity of NuBCP-9 in multiple mouse models is encouraging (Gupta et al. 2018; Kolluri et al. 2008; Kumar et al. 2014). BCL-2 functional conversion will likely be effective also on haematological malignancies which could provide a therapeutic option for refractory CLL. The discovery of the short peptide, NuBCP-9, and its effective delivery in vivo using nanoparticles identifies a promising new approach to cancer therapeutics.

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