

Locked Nucleic Acids Technology for Targeting Survivin in Cancer and Viral Infections

Jagat R. Kanwar* and Kislay Roy

Nanomedicine-Laboratory of Immunology and Molecular Biomedical Research (LIMBR), Centre for Biotechnology and Interdisciplinary Biosciences and Institute for Frontier Materials (IFM), Deakin University, Victoria, Australia

Survivin which is a member of inhibitor of apoptosis (IAP) family of proteins is known to play a key role in cell division and in apoptosis. It is found mainly in G2/M phase and very low in G0/G1 and S phase of the cell cycle [1]. Survivin has been found to be highly upregulated specifically in most cancer forms and absent in normal tissues, thus it serves as an important biomarker for tumour diagnosis and treatment [2,3]. Survivin modulates cell signalling pathways involving Wnt/ β -catenin, STAT3, TNFR, P13K/AKT, mTOR, HIF-1 α , NF- κ b, Erb-2, HSP90 and SPS thus having a key regulatory role in tumour progression [4]. It has been reported that survivin knockdown in tumour cells led to cell cycle arrest and apoptosis in tumour cells [5]. Survivin expression in cancer cells is not only related to cancer progression but it has also been reported that survivin expression co-relates with the drug resistance in B-cell lymphoma and treatments leading to survivin downregulation showed increased drug sensitivity in these cells. Thus, elevated levels of survivin expression have been correlated with drug resistance in cancer cells [6]. Studies have reported that viral infection also promotes survivin expression and increases the possibility of cancer. The latent membrane protein 1 (LMP1) of Epstein-barr virus (EBV) leads to an increase in P53 mediated survivin promoters activity and overexpression of survivin which can lead to nasopharyngeal carcinoma (NPC) [7]. The merkel cell polyomavirus (MCV) large T antigen interacts with the retinoblastoma protein (Rb) which is the cell cycle regulator protein and leads to overexpression of survivin that is responsible for 80% of all merkel cell carcinoma (MCC), a form of skin cancer [8]. The varicella-zoster virus that causes chicken pox triggers the signal transducers and activators of transcription 3 (STAT3) phosphorylation in cells which further induces survivin overexpression and may lead to development of cancer [9]. Role of survivin has also been explored in immune response and it was reported that the T cell cultures showed a decreased T cell proliferation and reduced cytotoxic T cell response post treatment with survivin. The reason behind this is that survivin induces a shift in the T cell response from type 1 to type 2 response with decrease in the cytotoxic T cell functioning [10].

Many therapies have been developed for survivin targeting in order to treat various forms of cancer. Survivin antagonists have been highly successful against established tumours as they have proven to downregulate survivin expression and induce apoptosis in cancer cells [11]. The advantage of using the dominant negative mutant is that they do not harm the normal body cells and target only the cancer cells [12]. Gene therapy against survivin also proved to be highly successful in mouse model of EL-4 thymic lymphoma cells [13]. The most recent targeting methodology is use of locked nucleic acids (LNA) modified aptamers, mRNA sequences and antisense-oligonucleotides which have much higher target specificity and have been more successful than other forms of targeting methods. The introduction of aptamers and LNA has brought a revolutionary change in the modern approach of targeting and diagnostics. Aptamers are ss DNA or RNA oligonucleotides which have the ability to fold into unique 3 dimensional (3D) conformations. These molecular ligands are highly selective and specific towards their targets and have a potential to inhibit target proteins in a broad range

of disease [14]. LNA are a new class of high affinity cyclic analogs of nucleic acids in which the furanose ring of ribose sugar is chemically locked in an RNA mimicking conformation by introduction of the 2'-O, 4'-C-methylene bridge and the conjugation of LNA with nanoparticles is known as locked nucleic acid nanoparticle conjugates of LNP's [15]. LY2181308 [16], YM155 [17], SPC3042 [18] and EZN-3042 [19] are some of the antisense molecules which have been used as survivin inhibitors. The cytotoxic and apoptotic effects of LNA modified siRNA to survivin encapsulated in chitosan coated ceramic anti-cancer nanocarriers has been reported. It was observed that these nanocarriers were effective in inducing apoptosis and cytotoxicity specifically to the colon cancer cells (Caco-2) whereas no significant cytotoxicity or apoptosis was observed in the normal intestinal cells (FHS). The *in vivo* bio distribution study of these nanocarriers disclosed that the maximum number of nanocarriers internalized in the tumour, liver, brain and intestine apart from low accumulation in spleen, kidney, lung and heart. Once these nanocarriers were targeted using LNA-modified epithelial cell adhesion molecule (EpcAM) aptamer a ligand against surface molecule highly expressed by cancer cells, the accumulation in tumour was nearly 9 fold higher when compared to other organs. Thus the aptamer based survivin inhibitors were effectively able to target and inhibit colon cancer cells both *in vitro* and *in vivo* [20]. The significance of survivin as a biomarker for cancer is evident and due to its specific overexpression in cancer cells it has become an important anti-cancer therapeutic target. Survivin targeting has been accomplished by use of antibodies, antisense peptides, carrier peptides, aptamers, antisense oligonucleotides and nanoparticles but none of the therapies have been as successful as the antisense LNA therapy against survivin. Two such forms of treatments have made it to the clinical trials and have proved to be very promising as the future medicine for treatment of cancer.

References

1. Kanwar RK, Cheung CH, Chang JY, Kanwar JR (2010) Recent advances in anti-survivin treatments for cancer. *Curr Med Chem* 17: 1509-1515.
2. Baratchi S, Kanwar RK, Kanwar JR (2010) Survivin: a target from brain cancer to neurodegenerative disease. *Crit Rev Biochem Mol Biol* 45: 535-554.

*Corresponding author: Dr. Jagat R. Kanwar, Associate Professor of Immunology and Cell Biology, Nanomedicine-Laboratory of Immunology and Molecular Biomedical Research (NLIMBR), Institute for Frontier Materials (IFM), Deakin University, Geelong, Geelong Technology Precinct (GTP), Deakin University, Pigdons Road, Waurn Ponds, Geelong, Victoria 3217, Australia, Tel: 0061-3-5227-1148; Fax: 0061-3-5227-3402; E-mail: jagat.kanwar@deakin.edu.au

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3. Cheung CH, Chen HH, Cheng LT, Lyu KW, Kanwar JR, et al. (2010) Targeting Hsp90 with small molecule inhibitors induces the over-expression of the anti-apoptotic molecule, survivin, in human A549, HONE-1 and HT-29 cancer cells. *Mol Cancer* 9: 77.
4. Kanwar JR, Kamalapuram SK, Kanwar RK (2011) Targeting survivin in cancer: the cell-signalling perspective. *Drug Discov Today* 16: 485-494.
5. Kanwar JR, Kamalapuram SK, Kanwar RK (2010) Targeting survivin in cancer: patent review. *Expert Opin Ther Pat* 20: 1723-1737.
6. Bhattacharyya J, Mihara K, Ohtsubo M, Yasunaga S, Takei Y, et al. (2012) Overexpression of BMI-1 correlates with drug resistance in B-cell lymphoma cells through the stabilization of survivin expression. *Cancer Sci* 103: 34-41.
7. Guo L, Tang M, Yang L, Xiao L, Bode AM, et al. (2012) Epstein-Barr virus oncoprotein LMP1 mediates survivin upregulation by p53 contributing to G1/S cell cycle progression in nasopharyngeal carcinoma. *Int J Mol Med* 29: 574-580.
8. Arora R, Shuda M, Guastafierro A, Feng H, Toptan T, et al. (2012) Survivin is a therapeutic target in Merkel cell carcinoma. *Sci Transl Med* 4: 133ra56.
9. Sen N, Che X, Rajamani J, Zerboni L, Sung P, et al. (2012) Signal transducer and activator of transcription 3 (STAT3) and survivin induction by varicella-zoster virus promote replication and skin pathogenesis. *Proc Natl Acad Sci U S A* 109: 600-605.
10. Jutzy JM, Khan S, Asuncion-Valenzuela MM, Milford TA, Payne KJ, et al. (2012) Tumor-Released Survivin Induces a Type-2 T Cell Response and Decreases Cytotoxic T Cell Function, *in Vitro*. *Cancer Microenviron* .
11. Cheung CH, Sun X, Kanwar JR, Bai JZ, Cheng L, et al. (2010) A cell-permeable dominant-negative survivin protein induces apoptosis and sensitizes prostate cancer cells to TNF- α therapy. *Cancer Cell Int* 10: 36.
12. Baratchi S, Kanwar RK, Cheung CH, Kanwar JR (2010) Proliferative and protective effects of SurR9-C84A on differentiated neural cells. *J Neuroimmunol* 227: 120-132.
13. Kanwar JR, Shen WP, Kanwar RK, Berg RW, Krissansen GW (2001) Effects of survivin antagonists on growth of established tumors and B7-1 immunogene therapy. *J Natl Cancer Inst* 93: 1541-1552.
14. Kanwar JR, Mohan RR, Kanwar RK, Roy K, Bawa R (2010) Applications of aptamers in nanodelivery systems in cancer, eye and inflammatory diseases. *Nanomedicine (Lond)* 5: 1435-1445.
15. Kanwar JR, Roy K, Kanwar RK (2011) Chimeric aptamers in cancer cell-targeted drug delivery. *Crit Rev Biochem Mol Biol* 46: 459-477.
16. Koshkin AA, Singh SK, Nielsen P, Rajwanshi VK, Kumar R, et al. (1998) LNA (locked nucleic acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* 54: 3607-3630.
17. Yamauchi T, Nakamura N, Hiramoto M, Yuri M, Yokota H, et al. (2012) Sepantronium Bromide (YM155) induces disruption of the ILF3/p54(nrb) complex, which is required for survivin expression. *Biochem Biophys Res Commun* 425: 711-716.
18. Hansen JB, Fisker N, Westergaard M, Kjaerulff LS, Hansen HF, et al. (2008) SPC3042: a proapoptotic survivin inhibitor. *Mol Cancer Ther* 7: 2736-2745.
19. Sapra P, Wang M, Bandaru R, Zhao H, Greenberger LM, et al. (2010) Down-Modulation of Survivin Expression and Inhibition of Tumor Growth In Vivo by EZN-3042, A Locked Nucleic Acid Antisense Oligonucleotide. *Nucleosides Nucleotides Nucleic Acids* 29: 97-112.
20. Kanwar JR, Kanwar RK, Mahidhara G, Cheung CHA (2012) Cancer targeted nanoparticles specifically induce apoptosis in cancer cells and spare normal cells. *Aust J Chem* 65: 5.