



Supervisor: - Associate Professor Jagat Kanwar

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Research Interests: Associate Professor Jagat Kanwar is an immunologist and molecular biologist. Before moving to Australia his research activities at the University of Auckland, New Zealand during the past decade have focused on devising new treatments mainly for cancer and autoimmune disease multiple sclerosis and inflammatory diseases such as asthma and inflammatory bowel disease (IBD). In Australia, his research has focused on exploring the roles of molecular mediators, antioxidants and cellular communication in the pathophysiological mechanisms of inflammatory diseases, including cancer. He is working on nanotechnology based peptide, siRNA and miRNA delivery for targeting survivin (currently most attractive cancer target), HIF-1 α and apoptotic cell signalling molecules expression in the colon, retinoblastoma and breast cancers. For commercial funded grants his research group carries out research in the areas of bioactives as immunomodulators, their role in chronic inflammation such as osteoarthritis. His publications have added to the body of knowledge in the fields of cancer gene therapy, cell biology, immunology and nanobiotechnology. Kanwar's research work generated in total of 8 patent/PCTs with two provisionals in preparation. Five of these patents have been licensed for commercialization to biotech companies Antisoma, NeuronZ, Neuren Pharmaceuticals and Fonterra.

Research platforms:

Nano-medicine, Molecular Biology, Gene Therapy, Molecular Immunology.

(A) Projects on cancer

1. Title: Investigate whether *let-7* miRNA is the master regulator of cell proliferation pathways and regulate the survivin gene expression in tumors angiogenesis

Since they were first discovered in 1993, miRNAs have created a great deal of excitement. MicroRNAs/miRNAs are noncoding short, 19 to 25 nucleotide RNA molecules. The expression of microRNAs is highly specific for tissue and developmental stage, and involved in numerous cellular processes including development, differentiation, proliferation, apoptosis and response to stress. It was shown that microRNAs inhibit the expression of protein encoding genes at the post-transcriptional level in a variety of eukaryotic organisms and reduce the levels of their target transcripts as well as the amount of protein encoded. MicroRNAs mediated tumorigenesis results from either down regulation of tumor suppressor genes or up regulation of oncogenes. Let-7 microRNA appears to be a master regulator of cell proliferation pathways. Let-7 miRNAs play important roles in animal development, cell differentiation, and metabolism and have been implicated in human cancer. This project will provide evidence whether let-7 functions as a tumor suppressor in cancers. This work will reveal whether the let-7 microRNA to be a master regulator of cell proliferation pathways in cancers.

2. Title: To study the effect of cell permeable negative survivin (CPDNSur) protein antagonist on the apoptotic and localization of survivin splice variants.



Cancer cells express survivin, which plays an important role in their proliferation. Based on the recent evidence which have indicated that multi-sub cellular localization of survivin is consistent with its multiple functions in cellular metabolisms and responses. The survivin splice variants/isoforms appear to have unique sub cellular localizations and functions as well. We have found that the dominant negative survivin gene therapy induced apoptosis (programmed cell death) and cytotoxic T cell activity (CTL) in tumors (*Kanwar et al. Journal of the National Cancer Institute, Vol. 93, 2001*). Recently we also reported that a cell-permeable dominant-negative survivin (CPDNSur) protein antagonist induces cancer cell death through caspase dependent and independent apoptosis (*Cheung CGA, Kanwar JR and Krissansen GW European Journal of Cancer 4:149. 2006*). In the present project we will study the effect of intracellular delivery of the cell permeable DNSurR9 on the expression of survivin its splice variant forms and sub-cellular localization of the survivin. The ability DNSurR9 to inhibit the expression survivin at the site of mitochondria/cytoplasm/nucleus levels. The role of cell-permeable (CPDNSur) as a competitive antagonist to investigate the molecular mechanisms by which wild-type survivin inhibits apoptosis will be explored.

3. Title: To study the efficacy of cell permeable negative survivin (CPDNSur) protein antagonist in reversing the chemo-resistance/ radio-resistance

Co-supervisor:-Dr Rupinder Kanwar

Recently, several reports have demonstrated that survivin expression plays an essential role in drug resistance and radio resistance, and that genetic or pharmacological modulation of survivin expression affects drug or radiation effectiveness in apoptosis induction. The objective is based on hypothesis that treatment of human breast, colon and retinoblastoma cell lines with cell permeable CNDNSur protein antagonist will not only inhibit tumor cell growth but also enhance tumor radiosensitivity by antagonizing the biological function of wild type survivin. It may degrade the expressed survivin protein and its splice variants in these tumor cells thereby making them chemosensitive and radiosensitive. In this project we will study the effect of recombinant CPDNSur protein antagonist on the survivin expression in the radiation exposed and commercially available anti-cancer drug (taxol, doxorubicin and carboplatin) exposed different tumor cells in tissue culture based assays.

4. Title: Targeting hypoxia-inducible factor 1 (HIF-1 α) using its antagonist loaded nanocarriers to enhance liver cancer therapies

The **broad aim of the present study** is to design chitosan-coated nanocores (CNC) for effective loading and protection of cell permeable HIF-1 α antagonists. To increase the bioavailability, conformational stability, and activity of these proteins/peptides, we will load them into chitosan-coated ceramic nanocores and encapsulated them into alginate gel for effective protection, mucoadhesiveness, and sustained release. In protein/peptide carrier selection, natural polymers such as chitosan and alginate will be used because they are biosafe; highly inert towards protein drugs; do not need organic solvents; and possess properties of mucoadhesiveness, biodegradability, low toxicity, low immunogenicity, ready availability, and inexpensiveness. Given the role of HIF-1 α in angiogenesis, glucose utilization and tumor-cell survival, and its association with poor prognosis in HCC, the proposed project aims to seek novel potent strategies by targeting HIF-1 α with our already established system. The HIF-1 α molecular targets will be firstly tested in several



HCC cells lines under hypoxic condition, and sequentially in xenograft animal models of HCCs alone or in combinational forms with conventional therapies.

5. Title: Tumor suppression by targeting survivin expression and function using survivin antagonists loaded nanocarriers

Co-supervisor:-Dr Rupinder Kanwar

We hypothesize that treatment of human breast and colon cancers, both *in vitro* and *in vivo*, with our fusion/recombinant cell permeable dominant negative (Cys84Ala) survivin (DNSurR9) protein antagonist and a peptidomimetic based combined survivin and heat shock protein-90 (HSP-90) antagonist, shepherdin when loaded on alginate gel-encapsulated, chitosan ceramic nanocores (ACNC) will induce apoptosis, inhibit cell proliferation, tumorigenesis and angiogenesis. The cell permeable survivin protein and shepherdin antagonists will render these tumors chemosensitive, radiosensitive by disrupting the cross talk between survivin and hypoxia inducible factor -1 α (HIF-1 α) and later corresponding expression and function of angiogenic factors such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). The proposed nanocarriers based therapy will have the ability **1**) to combat of the aggressive human cancer types where survivin overexpression is linked to poor prognosis, drugresistance and radioresistance and **2**) fortify other conventional radiotherapy and chemotherapeutic treatments commonly used against these cancers.

6. Title: Anti-tumour and anti-angiogenic activities of neem and Fe/Se-saturated lactoferrin: a potential alternative medicine

Co-supervisor:-Dr Rupinder Kanwar

Central hypothesis of the project is based on 1) the literature and etano-pharmacological evidence of antitumour activities and chemopreventive potential of Australian neem (*Azadirachta indica*) leaf extract preparations and 2) our recent *in vitro* and *in vivo* research publication/patents on the synergistic efficacy of iron-saturated bovine lactoferrin (Lf+) supplemented diet in combating lymphoma (EL-4), lung cancer (Lewis lung carcinoma), and melanoma (B16) tumours. We hypothesize that neem leaf extract used in combination with either iron-saturated bovine lactoferrin (here designated Fe-bLf+) or selenium saturated bovine lactoferrin (Se-bLf+) preparation will be more potent and effective in inhibiting the tumour growth and angiogenesis in both *in vitro* and *in vivo* xenograft models of human colon and breast cancers. We seek to study the efficacies of *Azadirachta indica* (neem) leaf extract alone and in combination with either Fe-bLf+ or Se-bLf+ preparation for the treatment of human breast and colon cancers. The focus of the project will be to elucidate on a biochemical and molecular basis the preclinical efficacies of the different treatments in targeting tumor cell growth and angiogenesis. The specific aims will be:

(B) Projects on neurodegeneration

7. Title: Therapeutic potential of Epstein-Barr virus (EBV) oncogene BARP1 protein as a neuroprotection: targeting neural cell apoptosis



Co-supervisor:-Dr Rupinder Kanwar

We and others have reported the significant increase in apoptosis (programmed cell death) in neurodegenerative diseases such as multiple sclerosis, Alzheimer's and neurotraumatic patients as well as in animal models. The apoptosis was induced in the nerve cells and oligodendrocytes of the central nervous system (brain and spinal cord) in these patients. Oligodendrocytes are myelin making cells which protect the neuronal cells from various insults such as glutamate, autoreactive T-cells, oxidative stress and inflammatory & pro-inflammatory cytokines. Recently BAF1 gene has been cloned and well characterized. In this project we will study the neuroprotective effects of BAF1 green fluorescent labeled protein (GFP) in the neural cells and oligodendrocytes by transfecting these cells with BAF1-GFP and GFP control gene which may accelerate the neuroregeneration processes of brain cell repair in neurodegenerative cell based assays and *in vivo* animal models. We will compare our results with our previously reported FDA approved neuroprotectors, AMPA/kainate antagonist NBQX, and the NMDA receptor antagonist GPE commercially known as "Glypromate" which is in clinical phase III trials (Kanwar et al Patents).

8. Title: Development of a novel cell based model mimicking human brain anatomy to study neural repair in multiple sclerosis

Co-supervisor:-Dr Rupinder Kanwar

Multiple sclerosis (MS) is a devastating non-neurotraumatic incurable chronic inflammatory disease. From the national perspective, pressure for MS research weighs heavy with the incidence increasing by 8% each year, affecting young Australians between 20 and 40 who are in their productive years in terms of family and career. The exact cause of MS is not known and there is no single drug that can cure MS. Scientific evidence suggests autoreactive T cells mainly responsible for the observed demyelination and death of neurons, oligodendrocytes and axonal damage (a hallmark in the pathogenesis of MS). Degenerated oligodendrocytes and neuronal cells in the brains of MS patients are unable to regenerate in order to repair the loss. Recently a role of survivin expression (an attractive cancer target) by autoreactive T cells has been reported in the death of neurons, oligodendrocytes and axonal damage. The current proposal seeks to develop a novel realistic model of human brain tissue repair *in vitro* by growing together in a co-culture well characterized human neuronal oligodendrocytic and astrocytic precursor cells. The neuroprotective efficacy of a cell permeable survivin wild type and dominant mutant negative molecules will also be studied.

(C) Projects on AIDS

9. Molecular diagnostics and related biomarker in HIV-infected patients.

Co-supervisor:- Dr. Ashok Chauhan, Associate Professor, School of Medicine, University of South Carolina, Columbia, SC 29208.



HIV infection and its treatment: Over 25 million people have died since the first case of AIDS was identified in 1981, and the number of people living with human immunodeficiency virus (HIV) worldwide continues to expand—from 35 million in 2001 to 40 million in 2006 (UNAIDS/WHO, 2006). Currently 27 antiviral therapies have been approved for use in HIV-infected patients (US FDA, 2007), including nucleoside, nucleotide, and nonnucleoside reverse transcriptase inhibitors, protease inhibitors, a chemokine receptor-specific entry inhibitor, and a fusion inhibitor. The first drugs approved to treat HIV infection inhibited the specific activity of the virally encoded reverse transcriptase (RT), the viral enzyme essential for conversion of the viral RNA genome into a DNA that integrates into the host genome. Despite the availability of effective screening systems for viral infection, it is not yet known which biomolecules (biomarkers) come into interplay as a part of HIV-infectivity, host response and HIV- disease progression. Since HIV-infection induces dysregulation of host physio-molecular profile and there is paucity of information on biomarkers in HIV-infection in Australia due to new immigration and globalisation. It is essential to pinpoint / target the key regulatory biomolecules that could be playing a pivotal role in HIV-infection and disease progression.