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We have developed a unique program within our laboratory to investigate cancer progression and metastasis; addressing the deregulation of inter- and intra-cellular signalling pathways in tumour cells. Our primary research focus is to determine molecular alterations implicated in promoting the invasive phenotypes of cancer cells.

To establish an understanding of molecular mechanisms promoting metastasis we have used a variety of cancer cell line models including both paediatric and adult cancers. Specifically, our research is focused on protein products of three genes: E-cadherin (CDH1), adenomatous polyposis coli (APC) and beta-catenin (CTNNB1). All gene products are components of the adherens junction. Besides their role in cellular architecture, all molecules have roles in *Wnt* signalling where beta-catenin functions as a transactivator for the expression of numerous cancer promoting genes. E-cadherin (E-cad) is indirectly involved in the modulation of β-catenin (β-cat) transcriptional function and APC is a negative regulator of the *wnt* pathway; a critical player in β-cat destruction machinery. β-cat is a very powerful cancer promoting protein. By studying the alterations that occur with β-cat during cancer initiation and progression, we will have a better understanding of the fundamental pathological changes that occur during cancer. We expect that knowledge gained from these studies will aid the improvement of cancer diagnostics and hence the quality of life of cancer patients; as it would facilitate early detection and characterize potentially aggressive tumours.

1. Role of a switch in cadherin phenotype and intracellular levels of β-cat in epithelial-mesenchymal transition (EMT):

Tumour progression proceeds from an early neoplastic lesion to a primary tumour *in situ* and finally, to a malignant tumour with the capacity to metastasize. This well coordinated process includes several steps: 1) the loss of intercellular cohesion, 2) the disruption of the extracellular matrix, 3) the modification of the cytoskeleton, and 4) the increased motility and invasion of the cancer cell. The latter is representative of a process referred to as EMT. EMT relies on the decrease or loss of cell-cell contact. Intercellular adhesions in both epithelial and mesenchymal cells are comprised of desmosomes, adherens junctions and tight junctions. In adherens junctions, E-cad links intracellularly to a complex containing beta-, alpha- and p120-catenins. In turn, this complex interacts with the actin cytoskeletal network. During the process of EMT the activity at the adherens junction is highly modified due to the loss of E-cad and its replacement by N-cadherin (N-cad). This switch in cadherin profile results in the destabilization of the cell-adhesion complex. Aberrant expression of N-cadherin is a prominent cause of increase in tumour cell motility, and hence migration and invasive potential. In a variety of cancers, loss of E-cad is linked to poor prognosis, tumour progression and metastasis. In addition to E-cad's role in adhesion, E-cad is also an important signalling molecule by virtue of its interaction with β-cat. β-cat is a multifunctional armadillo repeat protein regulated by the Wnt signal transduction pathway. When β-cat is released in the cytosol, it is phosphorylated in a complex containing APC, Axin and glycogen synthase kinase (GSK)-3β. This complex is responsible for β-cat's degradation through the ubiquitin-

proteasome pathway. Upon activation of Wnt signaling, phosphorylation of β-cat is inhibited, allowing β-cat to accumulate in the cytosol and subsequently translocate to the nucleus. Here, β-cat acts as a transactivator with T-Cell Factor (TCF) family of transcription factors to initiate the transcription of genes involved in cell survival, proliferation, differentiation, migration, invasion and metastasis. Elevated cellular expression, especially nuclear expression, of β-cat is a marker associated with poor prognosis in many adult and pediatric cancers. However, while the Wnt/β-cat pathway has been implicated in tumorigenesis, little is known regarding its involvement in tumor progression. In our laboratory, we seek to determine the involvement of these two integrated phenomenon, loss of E-cadherin & activation of Wnt/β-cat pathway in tumor progression. We expect our research to establish an understanding of novel mechanisms involved in promoting key molecular alterations during cancer progression.

2. The role of O-GlcNAc post-translational modification of β-cat in cancer progression: β-cat was recently identified to be modified by beta-N-acetylglucosamine (O-GlcNAc) post-translational modification. This involves an O-linked attachment of an O-GlcNAc moiety to specific serine and/or threonine residues. We initially worked with prostate and breast (normal and cancer) cell lines to evaluate whether O-GlcNac modification of β-cat regulates the signal transduction properties of the protein. We compared the O-GlcNAc of β-cat in normal prostate and breast cells to that of prostate cancer (CaP) and breast cancer (BCa) cell lines. We found significantly more O-GlcNAc β-cat in the normal cells compared to CaP and BCa cells. Furthermore, we found that O-GlcNAc of β-cat negatively regulates β-cat transcriptional activity. This is at least partially due to the negative regulatory effect of O-GlcNAc on the nuclear localization of the protein (Sayat et al. 2008). We have further determined that O-GlcNAc modification of β-cat causes the protein to localize predominantly to the plasma membrane.

We hypothesize that O-GlcNAc associated alterations in the cellular interactions of β-cat regulates β-cat's subcellular (nuclear/cytoplasmic/plasma membrane) localization and transcriptional activity. In this project we aim to gain an understanding of the mechanism by which O-GlcNAc of β-cat regulates nuclear localization and transcriptional function. A second aim is to determine the significance of O-GlcNac modification of β-cat in cancer progression.