

UPDATE OF RESEARCH AT THE PROTEOMICS CENTER AT CHILDREN'S HOSPITAL BOSTON

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My laboratory is engaged in the study of the molecular mechanisms underlying the role of cholesterol in human degenerative diseases related to bone, including prostate cancer (bone metastasis being a serious consequence of malignant disease), osteoporosis, and vascular calcification (part of the sequelae of atherosclerosis).

One of the ways we believe that cholesterol affects both normal and pathological cellular processes is through its role in the formation of structures referred to as lipid rafts. Lipid rafts are membrane domains that due to the combined properties of cholesterol and fatty acids with long saturated acyl chains (e.g. those found in sphingolipids like the GM1 ganglioside) are in a liquid-ordered phase, in contrast to the majority of the cell membranes, which are in a liquid disordered phase. These membrane substructures regulate growth factor receptors in bone forming cells (osteoblasts and pericytes) and in prostate tumor cells. Specifically we have demonstrated that platelet derived growth factor (PDGF) receptors are regulated by lipid rafts in osteoblasts and pericytes, while epidermal growth factor receptors and the intracellular cell survival factor AKT (aka protein kinase B) are regulated by lipid rafts in prostate tumor cells.

Using multiple approaches, in a variety of mesenchymal cell types (osteoblasts, pericytes, smooth muscle cells), and other experimental systems (zebrafish) we have begun to untangle the critical central from the less important peripheral functions of raft domains. We have been able to demonstrate that lipid rafts are sites where 1) receptor signaling causes an increase in components of the actin cytoskeleton and its regulators (using an unbiased quantitative proteomics approach), 2)

that diaphanous (a member of the formin family of actin regulators) functions within raft domains, 3) that suppression of the expression of caveolin (a structural component of a type of raft domain referred to as caveolae "little caves") leads a lethal collapse of the actin cytoskeleton, 4) and that formation of cell extensions (e.g. pseudopodia) originate in lipid raft domains in response to growth factor (PDGF)-induced signaling.

We have also initiated pioneering studies examining the role of cholesterol in degenerative disease using in vivo animal models. We have been able to demonstrate that elevated serum cholesterol leads to accelerated growth of implanted tumors (human tumors xenografts implanted into SCID mice), through a lipid raft-mediated mechanism involving increased AKT activity and a suppression of apoptosis. We have also shown that elevated serum cholesterol causes decreased bone mineral density specifically in the femoral head of experimental animals.

Lastly, we are deeply involved in unraveling the molecular mechanism behind the effect of statin drugs (HMG-CoA reductase inhibitors, which block cholesterol formation by blocking an early step in its synthesis pathway) on apoptosis (in prostate tumor cells and pericytes, but not in normal prostatic ductal epithelial cells and osteoblasts) and proliferation (causes decreased proliferation of osteoblasts). We find that statin drug effects are reversible by cholesterol addition to cell membranes, others have reported that statin drugs function in these respects by inhibiting prenylation of critical signaling molecules such as the Ras, Rac, Rho family of GTPases. We are exploring whether one or both of the mechanisms are responsible for the pleiotropic effects of the statin drugs.

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Selected Publications

- Solomon***, K.R., **Danciu, T.E.**, **Adolphson, L.D.**, **Hecht, L.E.**, and **Hauschka P.V.** Caveolin-enriched membrane signaling complexes in human and murine osteoblasts. *J. Bone Miner. Res.* 15:2380-2390, 2000.
- Zhuang, L.**, **Lin, J.**, **Lu, M.L.**, **Solomon#***, K.R., and **Freeman#***, M.R. Cholesterol-Rich Lipid Rafts Regulate Growth Factor-Activated Akt Signaling and Cell Survival in Human Prostate Cancer Cell. *Cancer Res.* 62:2227-2231, 2002.
- Solomon, K.R.**, **Sharma, P.**, **Chan, M.**, **Morrison, P.T.**, and **Finberg, R.W.** CD109 represents a novel branch of the $\alpha 2$ -macroglobulin/complement gene family. *Gene* 327:171-183, 2004.
- Zhuang, L.**, **Kim, J.**, **You, F.**, **Adam, R.M.**, **Solomon, K.R.**, and **Freeman, M.R.** Cholesterol targeting alters lipid raft compositions and cell survival in prostate cancer cells and xenografts. *J. Clin. Invest.* (2005, In Press and on line March 17).
- McLellan, D.L.**, **Adam, R.M.**, **Steen H.**, **Gygi, S.P.**, **Garlick M. Freeman, M.R.** and **Solomon, K.R.** A Quantitative Proteomic Analysis of Growth Factor-Induced Compositional Changes in Lipid Rafts of Human Smooth Muscle Cells (Accepted, 2005, Proteomics).