"Mis-regulation of Protein Translation in the Pathogenesis and Treatment of Cancer"

Principal Investigator:
- Michael B. Yaffe, PhD, Broad Institute of MIT and Harvard

Co-Principal Investigators:
- Steven Carr, PhD, Broad Institute of MIT and Harvard
- Andrew Koff, PhD, Memorial Sloan-Kettering Cancer Center

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Abstract: In the last several years it has become increasingly apparent that the mis-regulation of protein translation plays a critical role in cell transformation, tumorigenesis, and the resistance of cancer cells to cytotoxic therapies. A wide variety of human tumors have been shown to depend on activated signal transduction pathways that control protein translation (i.e. PI 3-kinase and mTor pathways), and inhibition of mTor-driven protein translation by the drug rapamycin is now in clinical trials as a cancer treatment. Similarly, many human tumors over-express one or more translation initiation factors (i.e. eIF4E, 4G and 4A), and experimental overexpression of these proteins in non-transformed cell lines directly leads to cell transformation in culture and tumor formation in transgenic mouse models. However, the identities of many of the mis-translated proteins that contribute to oncogenesis, metastasis, and resistance to therapy are unknown, because until now there has been no unbiased method by which to identify them in cancer cells in a proteome-wide manner. In this proposal we will develop and optimize a novel technology – Bio-Orthoganol Non-Canonical Amino Acid Tagging – for the non-biased mass-spectrometry-based mapping of actively translated proteins (hereafter referred to as the 'translatome'). In the first phase of the pilot project we will analyze and optimize the capture of azidohomoalanine-tagged nascent proteins by click chemistry. In the second phase of the pilot project we will show proof-of-principle by using this approach to identify newly translated proteins in normal and cancerous human breast cells. In the third phase of the pilot project we will investigate whether mass spectrometry-based analysis of the captured proteins can provide rigorously quantitative information on translation rates, and whether the sensitivity of the approach can be improved, by combining BONCAT and SILAC (stable isotope labeling in cell culture) technologies. These pilot studies should ultimately pave the way for application of these techniques to identify mis-translated proteins in human cancer, as well as proteins whose translation is upregulated in cancers that show enhanced metastatic potential or resistance to chemotherapy and radiation treatment.