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TITLE: Targeting the MTA 1s-LM04 Pathway in Hormone Resistance

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**Re: Grant Award number W81XWH-04-1-0648 entitled, "
Targeting the MTA1s LMO4 pathway in hormone resistance"
TARGETING THE MTA1S-LMO4 PATHWAY IN HORMONE RESISTANCE**

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LIM domain transcriptional regulators are critical mediators of pattern formation, organogenesis and cell differentiation. The LIM-only proteins (LMO) consist nearly entirely of two LIM domains and utilize these cysteine-rich, zinc-coordinating regions to help dictate patterns of gene expression and cell fate through mediating protein-protein interactions with DNA binding proteins and transcriptional coregulators. In addition to their developmental roles, LMO proteins may also be critical mediators of cancer development. LMO4, the most divergent LMO protein, was originally cloned from a breast cancer cDNA library and is overexpressed in more than 50% of invasive breast cancers. While investigating the function of a new cytoplasmic protein, MTA1s (metastasis-associated protein 1 (MTA1) short form) in breast cancer, we have found that MTA1s physically interacts with LMO4. Cytoplasmic localization of LMO4 has been noted in late stage human breast cancers. Since MTA1s has been shown to contribute to the cytoplasmic localization of estrogen receptor alpha (ER), enhancement of nongenomic ER signaling, and the development of hormone-resistant breast cancer, we tested the hypothesis that LMO4 is a new ER coregulator that facilitates MTA1s-mediated ER cytoplasmic localization and nongenomic signaling. As a model system, we have developed paired tamoxifen-sensitive and tamoxifen-resistant, ER expressing breast cancer cells that over express LMO4, MTA1s, or both proteins. Routine biochemical, molecular biology and confocal microscopy techniques have been employed in these studies. Results indicate that LMO4 and ER physically interact in vitro and in vivo. Deletion mapping determined that the first 164 amino acids of MTA1s are required for this interaction, while both LIM domains of LMO4 are required for optimal protein-protein interaction. Using transient transfection of an estrogen response element (ERE)-luciferase reporter system, we determined that LMO4 is a potent suppressor of ER-mediated transcriptional activity. Likewise, treatment of ERE-luciferase transfected cells with siRNA directed against LMO4 resulted in a more than four fold increase in both basal and estrogen-induced reporter activity. Although both proteins showed significant cytoplasmic localization under different conditions using immunofluorescent labeling and confocal microscopy, most LMO4-ER colocalization appeared to be in the cell nucleus. Thus LMO4 appears to be an important regulator of ER function. This regulation may encompass both nuclear and cytoplasmic ER. .

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