

**NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM
DECEMBER 15 – 17, 2014
POSTER ABSTRACTS – SESSION 2 (DEC. 16, 2014)**

Life, death, and function: the primate-specific long non-coding RNA transcriptome

Awardee: Leonard Lipovich

Award: New Innovator Award

Awardee Institution: Wayne State University

The ENCODE (Encyclopedia of DNA Elements) Consortium has highlighted the extraordinary abundance of long non-coding RNA (lncRNA) genes in the human genome, while revealing through its Gencode catalog that protein-coding genes comprise less than one half of the human gene count. In contrast to the better-understood microRNAs, lncRNAs act through diverse, heterogeneous mechanisms, epigenetically and post-transcriptionally, as both positive and negative regulators of gene expression. A remarkable and largely unique property of lncRNAs is their low interspecies conservation: at least 5,000 human lncRNAs are not conserved beyond primates. With a custom high-density oligonucleotide microarray, we examined the estrogen responsiveness of 4,312 full-length lncRNAs in the human MCF7 estrogen receptor alpha (ER α) positive cell line, a key breast cancer model. We identified 127 significantly estrogen-regulated lncRNAs. RNAi of 15 estrogen-induced and overexpression of six estrogen-repressed lncRNAs demonstrated concordant post-perturbation phenotypes across six phenotypic assays: MTT (cell proliferation), trypan blue, BrdU incorporation, PARP western blots and TUNL (apoptosis), and crystal violet staining (cell count). Our estrogen-responsive lncRNAs, which had consensus ER α site sequences and ChIP-seq evidence for ER α binding, shifted breast cancer cells along the apoptosis-proliferation axis. Reduced cell growth and increased cell death were consistently observed upon RNAi of estrogen-induced, and overexpression of estrogen-repressed, lncRNAs. These functional lncRNAs frequently exhibited primate-specific exonic sequences, and relied on primate-specific splice sites and polyadenylation signals to delineate their gene structures and boundaries. One primate-specific estrogen-repressed lncRNA reduced ERK1 and 2 phosphorylation in two ER α positive breast cancer cell lines, consistent with the accompanying decrease in cell viability, and suggesting that non-conserved lncRNAs may provide regulatory inputs into the MAP kinase pathway. These lncRNAs likely originated after the prosimian split, and represent candidate novel targets for cancer therapeutics, because, as new outlier nodes linked by sparse edges to gene regulatory networks, they can be perturbed without impacting conserved network hubs. These lncRNAs' evolutionary histories expose the limitations of nonprimate animal models of cancer, and contribute to the genomic basis of human disease.

In the next phase of this work, I will extend the six functional assays to all 127 leads. I will employ second- and third-generation RNAseq, instead of microarrays, to impute the complete MCF7 estrogen-responsive lncRNAome. I will identify novel primate-specific lncRNAs from RNAseq data and subject them to the same system perturbations and phenotypic assays. Having demonstrated ectopic translation of specific lncRNAs within the framework of the ENCODE Consortium lncRNA proteogenomics effort, I will next test whether primate-specific functional lncRNAs act directly as RNAs, not via translated peptides, and will for the first time

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jointly use RiboSeq and mass spectrometry to determine whether human lncRNA ectopic translation is hormone-responsive. The results will address a fascinating new question with the potential to initiate a paradigm shift in cancer biology: Is human cancer, to an extent, a primate-specific disease caused by non-conserved lncRNAs? Implementation of this proposal will yield a conservation-unbiased, high-throughput assignment of cellular functions to primate-specific lncRNAs in a major nuclear hormone receptor pathway relevant to cancer therapeutics.

I very gratefully acknowledge the NIH Director's New Innovator Award program for supporting this research through 1DP2CA196375-01.