Symposium: ASAS Cell Biology: The Role of MicroRNA on Cell Function

161 MicroRNA: Mechanism of gene regulation. T. G. McDaneld*, *USDA/ARS U.S. Meat Animal Research Center*, *Clay Center, NE*.

MicroRNA (miR) are a class of small RNAs that regulate gene expression by inhibiting translation of protein encoding transcripts through activation of a specific cellular pathway. The small RNA classified as miR are short sequences of 18-26 nucleotide long, encoded by nuclear genes with distinctive properties that comprise 1-5% of known genes. During processing from the primary transcript, the mature miR sequence is loaded into an RNA:protein complex known as the "RNA induced silencing complex" (RISC). The sequence of the miR loaded in the complex targets the RISC to specific binding sites in the 3'UTR of mRNA transcripts, resulting in either degradation of the miR:mRNA complex or translocation to P-bodies. In either case, association of RISC with mRNA causes decreased translation of the targeted gene product. Approximately 40% of genes have transcripts that are potential targets for miR, suggesting that miR play an important role in multiple cellular processes. A single miR can target numerous distinct mRNA for decreased translation, and as a result miR appear to be intimately involved in developmental decisions including cell fate, cell cycle progression, apoptosis, adipocyte differentiation, and processes that alter muscle development and growth. Implication of miR in such a wide array of cellular processes has increased interest in evaluating miR in multiple biological models.

Key Words: MicroRNA, Gene Regulation

162 Role of MicroRNAs in hepatocarcinogenesis in an animal model. K. Ghoshal*, J. Datta, and H. Kutay, *Ohio State University*, *Wooster*.

In the post-genome era lot of effort has been expended to elucidate the function of noncoding regions of the genome that was previously considered as "junk DNA". Majority of these DNAs codes for RNAs ranging in size from ~100 to ~10,000 nucleotides. MicroRNAs (miRs) are ~21-nucleotide-long RNA that regulate expression of protein-coding genes by post-transcriptional mechanisms. Most of these miRs, highly conserved among animals, function by base pairing with the 3'-UTR of mRNA resulting in translational repression or mRNA degradation. MiR genes transcribed by RNA polymerase II, can be intergenic, intronic or polycistronic. Primary miRNAs are processed by ribonucleases to mature miRs. Like mRNAs, some miRs are ubiquitously expressed whereas others are tissue specific. Each miR targets several mRNAs, which in turn, are regulated by multiple miRs, underscoring their complex regulatory role. MicroRNAs are involved in every aspect of biology that include differentiation, development, immune response, drug resistance etc. Aberrations in microRNA expression appear to play a causal role in different disease processes including cancer. Studies from several laboratories have shown that microRNA signature is altered in primary hepatocellular carcinomas that can be used as a prognostic marker. In an animal model of diet-induced hepatocarcinogenesis we have shown that miR expression profile in the rodent liver tumors is quite similar to those in humans. Some miRs are deregulated an early stages of tumorigenesis implicating their role in preneoplastic transformation of hepatocytes. Currently we are pursuing potential role of some of the deregulated miRs in hepatocarcinogenesis.
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Key Words: MicroRNA, Liver Cancer, Tumor Suppressor Genes

163 MicroRNAs in the ovary and female reproductive tract. L. Christenson*, M. Carletti, S. Fiedler, L. Luense, and X. Hong, *University of Kansas Medical Center*, *Kansas City*.

Post-transcriptional gene regulation plays a vital role in male and female germ cell function, but our understanding of this regulatory process in somatic cells and its impact on reproductive tissue development and function is not understood. In mammalian cells, microRNAs (miRNA) are key post-transcriptional regulators and function primarily by modulating translation of their target mRNAs. Mature miRNAs are synthesized through a multi-step process that concludes with the cleavage of stem-loop pre-miRNAs by the RNase III enzyme, Dicer. To understand the role of miRNA mediated post-transcriptional gene regulation in somatic cells of the female reproductive tract and in ovarian granulosa cells, mice with loxP insertions in the Dicer gene were crossed with mice expressing Cre-recombinase driven by the anti-Mullerian hormone receptor-2 (AmhR2) promoter. Female $Dicer^{fl/fl}$;AmhR2-Cre mice (n=6) mated to fertile males failed to produce offspring. Mating was confirmed, thus estrus and presumably normal follicular estrogen synthesis occurred in these mice. General morphological and histological evaluation of these tissues indicated that the oviduct and uterus were half the size of their littermate controls and a severe disruption of the oviductal lumen at the utero-tubal junction was evident. Ovarian function was also not normal in the $Dicer^{fl/fl}$;AmhR2-Cre mice as evidenced by reduced ovulation rates and the relative absence of corpora lutea. To further understand the role of miRNAs in the ovulatory process, expression of ovarian granulosa cell miRNAs, before (0 h) and 4 h after the luteinizing hormone (LH/hCG) surge was evaluated. More than 200 miRNAs were detected in granulosa cells and 15 of these exhibited differential expression ($P < 0.05$). Three miRNAs, miRNA-21, -132 and -212 exhibited a pronounced (7.5 to 20-fold) up regulation in response to in vivo hCG treatment. Subsequent in vitro studies with granulosa cells and antagonists (i.e., anti-mirs and complementary LNA primers) to these specific miRNAs has implicated these miRNAs in regulation of apoptosis and general translation control. This work is supported by NIH grant HD051870.

Key Words: microRNA, Granulosa Cell, Ovary