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ABSTRACTS

Micro RNA and non-coding DNA and repeats

076: Noncoding RNAs in nervous system function

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In the more than 7 years since the first publications on the entire human genome, the number and general architecture of protein coding genes, comprising only some 1.2% of the genome, has remained remarkably stable. By contrast, surprising changes have come from noncoding regions, which have created an entirely new paradigm for all aspects of genome-related research. Several high-throughput efforts, by ourselves and others, have provided strong evidence that, in contrast to earlier understanding, a great majority of the mammalian genome is transcribed into noncoding RNAs. Why do organisms and perhaps the mammalian brain in particular, consume so much of its energy to transcribe all these RNA transcripts? And is this newly recognized universe of noncoding RNAs of functional (regulatory) importance? A primary objective of higher life forms is to gather larger amounts of more relevant information, and to apply that information toward the improvement of its future information-gathering ability. Noncoding RNAs with regulatory function may be critical in this regard. This presentation will highlight some of our recent examples of long and small noncoding RNAs with regulatory activities on specific genes and pathways (e.g., references 1–4).

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077: Quality control of miRNA in biological extractions

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In recent years, hundreds of microRNAs have been discovered. Although their functional roles remain to be classified in detail, accumulating experimental data suggest a fundamental involvement in cancer development and progression. The expression level of individual miRNAs is typically quantified by methods like northern blot, RT-PCR or microarray hybridization. One of the major drawbacks in miRNA research is the lack of adequate analytical methods for small RNA extractions before going in the subsequent analysis of the miRNA fraction. As different studies showed that microRNA profiles could be of value in cancer diagnosis, the quality control and quantification of miRNA samples becomes more and more important. Here we present a highly sensitive microfluidic 'high resolution' assay for the analysis of small nucleic acids. The assay utilizes a novel sieving matrix formulation and a special dye system which allows detecting miRNA fraction down to a concentration of 50 pg. It measures integrity, size and concentration of small RNA and is especially calibrated for miRNA. In conclusion, our approach allows the implementation of miRNA quality control in a miRNA expression workflow by monitoring miRNA fractions derived from different tissues or extraction methods.

078: Endogenous XIST complementary small RNA and RNAi machinery in human X dosage compensation

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Equalization of X-linked gene expression between two sexes despite their differences in copy number is mediated by transcriptional silencing of one X chromosome in female cells. Small regulatory RNA from the non-coding repetitive sequences plays crucial roles in transcriptional silencing and heterochromatin formation. To investigate the involvement of RNAi and small non-coding RNA in the maintenance of human X inactivation, we have performed strand-specific

northern blots using antisense/sense probes derived from XIST exon-1 with RNA extracted from different human male and female cell lineages. Small non-coding RNA species ranging from ~18 to 25 nt in length with predominant class of 21–24 nt RNA were observed. These RNA referred as sXiRNA (small XIST RNAs) were found in RNA from the different human cells carrying at least one active X chromosome. These small regulatory RNAs are independent to RNA polymerase II transcription and Xi localized XIST or TSIX transcripts. Further, preferential localization of sXiRNA in the nuclear fraction indicates their functional role in the nuclear transcriptional control rather than post transcriptional gene regulation. These small RNA are complementary to Repeat C region but not to other regions of XIST transcript. Formation of sXi RNA requires DICER1, an RNase III class enzyme, central mediator of RNAi pathway in human cells. DICER1 depletion in somatic cells leads to significant reduction in sXiRNA levels revealing the double stranded RNA (dsRNA) presence within the XIST locus of somatic cells. DICER1 depletion also leads to global and X chromosome specific increase in histone modifications indicative of transcriptionally active chromatin and increase in transcription of selected X linked genes from distinct X chromosomal loci. These effects were partially rescued by exogenous addition of sXiRNA indicating specific role of RNAi pathway and sXiRNA in the process of the Xi maintenance. Therefore, these repeat derived sXiRNA of XIST locus might be crucial players in mammalian RNAi mediated Xi transcriptional silencing complex by altering histone code and organization of silent X chromatin.

079: Role of transcriptional dysregulation in polyglutamine mediated neuronal cell death

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Expansion of CAG repeats in coding and non-coding regions of genes is associated with neurodegenerative disorders. In coding regions, the CAG repeat corresponds to a polyglutamine stretch in the protein. Polyglutamine expansion in the protein leads to formation of intranuclear protein aggregates and is correlated to neurodegeneration in specific regions of the brain. However, the molecular effects that lead to neurodegeneration have remained elusive. We have explored the role of transcriptional dysregulation by TATA-box binding protein (TBP) containing an expanded polyglutamine stretch in a mouse neuronal cell culture based model. Polyglutamine expansion in the N-terminal region of TBP is associated with a neurodegenerative disorder called Spinocerebrellar ataxia 17 (SCA17). We find that mouse neuronal cells expressing a variant of human TBP harboring an abnormally expanded polyQ tract not only form intranuclear aggregates, but also show global transcription dysregulation. We identified differentially expressed genes from cDNA and high-density oligonucleotide array platforms and validated the differential expression at different time-points using independent methods like real-time PCR and western. We have earlier shown that the differentially expressed genes include the Voltage dependent Anion Channel1 (VDAC1), whose overexpression has been shown to induce apoptotic cell death in other cell types. We monitored cytochrome c release overtime and found concomitant upregulation of VDAC1 and increase in cytoplasmic cytochrome c, followed by apoptosis. Overexpression of VDAC1 could also induce the same effects in neuronal cells. Further, we found that interferon induced genes and the signal transducer and activator of transcription (STAT1) gene were differentially expressed in cells expressing an expanded polyglutamine allele of TBP, suggesting the putative role of STAT1 and subsequent involvement of inflammation in SCA17.

The recent discovery of large number of small cellular RNAs, called microRNAs suggest that the transcriptome and proteome are significantly influenced by their post-transcriptional regulation. We explored changes in the expression profile of microRNAs in cells expressing an expanded polyglutamine allele of TBP, compared to a normal allele. The differentially expressed microRNAs and mRNAs fall into similar cellular pathways and provide a comprehensive view of mutually reinforcing pathways of cellular response in polyglutamine diseases.

080: Mechanistic insights into Kcnq1ot1 non-coding antisense RNA-mediated transcriptional silencing of the chromosomal domains

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Transcriptome studies over the past few years have revealed that the genomes of all studied eukaryotes are almost entirely transcribed and that a significant part of the mammalian genome codes for non-translated RNA (ncRNA) while only 1.2% of the mammalian genome codes for protein-coding RNA. It is increasingly apparent that many of these ncRNAs possess regulatory functions. NcRNAs can be classified into two groups; small ncRNAs (21–31 nucleotide long, and include siRNAs, miRNAs and PiRNAs) and long ncRNAs (range in size from 100 bp to several hundred kb). Interestingly, both small and long ncRNAs seem to regulate diverse functions through regulating chromatin architecture and epigenetic memory, thus underlining the functional role of ncRNA in the maintenance of chromosome structure and function. Of particular interest is that gene clusters, with parent-of-origin-specific expression patterns, often contain one or more ncRNAs as their partners. Some of these ncRNAs are very large, ranging in size from 50 kb to several hundred kb, and transcribed in antisense direction from an intronic portion of protein coding gene. The expression of long antisense ncRNAs has been shown to be correlated with the repression in *cis* of protein coding genes, spread over several hundred kb on either side of the antisense transcription unit, indicating a link between ncRNA expression and silencing of neighboring protein-coding genes. Over the last few years my laboratory has been working to understand the functional role of long antisense ncRNAs in transcriptional silencing using Kcnq1ot1 as an example. The Kcnq1ot1 promoter maps to a 3.6 kb imprinting control region (Kcnq1 ICR) in intron 10 of the Kcnq1 gene and is encoded only on the paternal chromosome, as the Kcnq1ot1 promoter is methylated on the maternal chromosome. It is transcribed in antisense orientation to the Kcnq1 gene. The production of Kcnq1ot1 RNA on the paternal chromosome has been linked to the silencing of nearly 10–12 genes spread over a one mega-base Kcnq1 domain. Here we show that Kcnq1ot1 is 91 kb long and contains a 0.9 kb silencing domain at its 5′ end, which mediates bidirectional silencing through positioning the flanking sequences in the perinucleolar compartment, during mid S phase. More importantly, it carries out lineage-specific silencing through regulating the chromatin by exploiting lineage-specific silencing mechanisms. Our data, therefore, provide mechanistic insights into epigenetic regulation by long antisense ncRNAs.

081: The human satellite III non-coding RNA is involved in general stress response and modulates the cellular toxicity of misfolded proteins associated with neurodegenerative disorders

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Nuclear stress granules (NSG) are sub-nuclear compartments formed in the human cell under the condition of heat shock. Under thermal stress, the heat shock transcription factor-1 activates the transcription of satellite III repeats (S3R) into stable RNAs that eventually form the NSG. S3R transcripts associate with several splicing factor and other RNA processing proteins, and thereby possibly protect them during stress conditions. In an effort to understand the cellular functions of this non-coding RNA, we have blocked or over-expressed S3R transcripts under various stress conditions in a cellular model and evaluated the effect on cell survival and functions. Our studies show that, besides the heat shock, the S3R transcription is induced by proteasomal blockade or by an increase in the cellular levels of misfolded proteins. While S3R knockdown leads to apoptosis in heat-shocked cells, its overexpression does not appear to have any adverse effect on the cell survival. Curiously, S3R overexpression elevates the toxicity caused by huntingtin protein with expanded polyglutamine repeats. S3R show intriguing similarity with hsr omega transcripts of Drosophila. Both are non-coding transcripts with repeat sequences, and are strongly induced under the condition of heat shock, suggesting that they are functional homologues. Consistent with this notion, overexpression of hsr omega transcripts rescued the loss of S3R under heat shock in human cells, and increased the toxicity of mutant huntingtin protein when expressed together. Intriguingly, hsr omega is known to increase the cytotoxicity of expanded huntingtin protein in fly models. Our findings, besides strengthening the notion that SatIII and hsr omega are functional equivalents, provide novel functions for noncoding RNAs in stress response and in disorders associated with toxic gain-of-function proteins. We will discuss the cellular pathways that are regulated by S3R and the factors that regulate the expression of S3R.