piRNome of human endometrioid ovarian carcinoma

ABSTRACT

PIWI-interacting RNAs (piRNAs) are a recently discovered class of small non-coding RNAs (ncRNAs) of 24–32 nts length initially identified in germlines. More recently, these ncRNAs have been detected in several human cancer cells as well as in somatic cells which suggest that piRNAs play diverse roles by controlling gene expression in these cells. In this study, we explored the expression and function of piRNA pathways in human endometrioid ovarian carcinoma (ENOCa). ENOCa is the second most common type of epithelial ovarian cancer, the most lethal gynecological malignancy ranking fifth in leading cause of cancer-related deaths among women. To date, effective screening strategies and biomarkers have not been developed for epithelial ovarian cancer. ncRNAs have been proven promising biomarkers for different cancer types. In this work we have investigated and identified the repertoire of piRNAs in ENOCa through small RNA sequencing (sRNA-seq) of post-operated tissue samples of this carcinoma and normal epithelial ovarian tissue. Our analysis revealed 3143 and 2194 known piRNAs in ENOCa and epithelial ovarian tissue respectively. The differential expression analysis showed 49 significantly expressed piRNAs in ENOCa compared to the normal control. Further investigations are underway to evaluate the role these piRNAs in specific biological processes leading to ENOCa.

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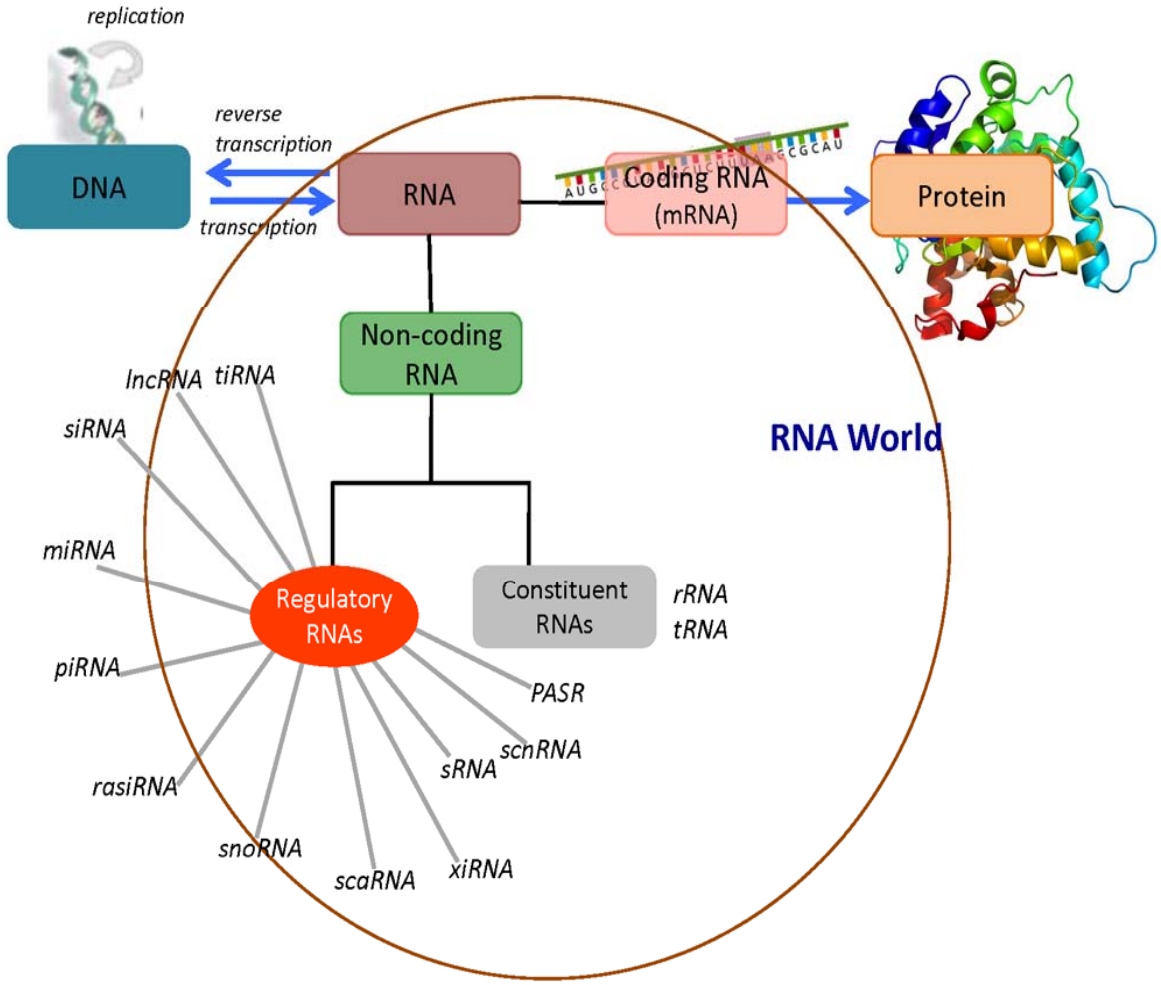
Regulatory non-coding RNAs (rncRNAs)

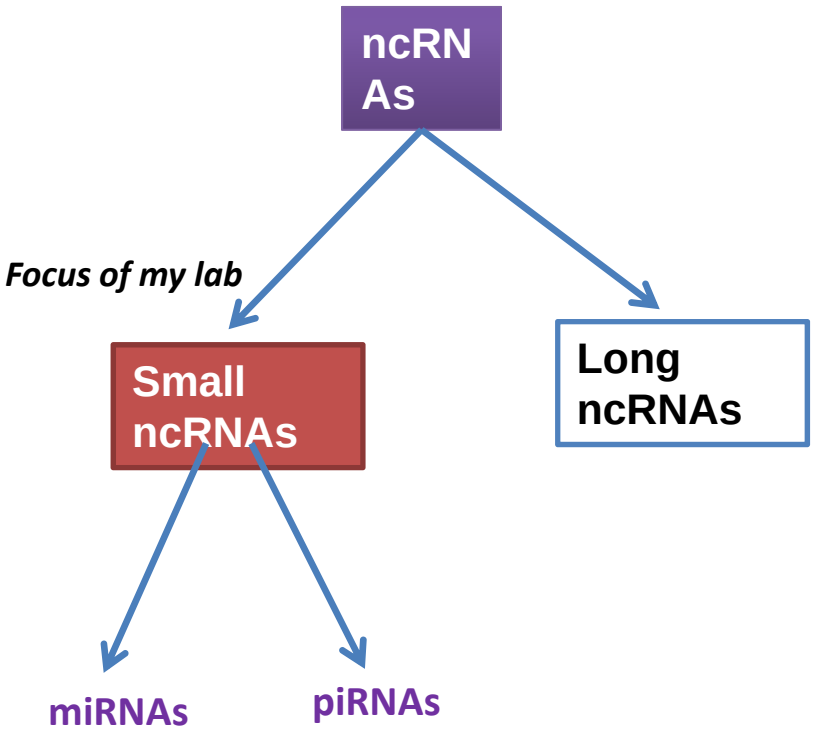
❖ **These are genomic "dark matter“ and their discovery continues due to advent of new technologies…..**

❖ **Encyclopedia of DNA Elements Consortium (ENCODE) projects revealed that eukaryotes transcribe up to 90% of their genomes, whose major fraction encodes ncRNAs with no coding ability and only a small fraction codes for proteins**

❖ **rncRNAs are involved in developmental processes including stem cell and germline maintenance, proliferation and differentiation and when dysfunctional, underpin diseases**



Based on length, ncRNAs are classified as follows:



miRNAs: These are about 18-25 nts in length that associate with Argonatute proteins and induce post-transcriptional gene regulation by binding primarily to 3'UTR of the targets through complementary base pairing.

piRNAs: Newly identified class of ncRNAs which are about 24-33 nts in length (first reported in 2006).
These associate with PIWI proteins and regulate transposon activity & regulate gene expression. Initially, these were thought to function only in germline development and gametogenesis. However, these are now reported in other cell types.

Why piRNAs (knowns of the unknown) ?

❖**piRNAs are generally 24–33 nucleotides in length and bind specifically to the PIWI subfamily of Argonaute proteins**

❖**piRNAs were initially discovered in germlines and thought to be restricted to these cells only to maintain germline genome integrity by repressing transposons. However, these are now reported in diverse cell types and function in the regulation of genes**

❖**Four human PIWIs: HIWI (PIWIL1), HILI (PIWIL2), HIWI2 (PIWIL4) and HIWI3 (PIWIL3)**

❖**The expression of PIWI and piRNAs are now reported in different cell types (both pluripotent & differentiated cells) as well as in few cancer types**

❖**Prof. KS Kosik & his group at University of California, Santa Barbara reported piRNAs in mouse hippocampus and elucidated their functions**

❖**Moreover, many facets of piRNAs and their mode of regulations/functions are unknown.** ***How these piRNAs interact with their target mRNAs? Is it similar to miRNA targeting ????***

❖ ***Are these are expressed in all tissues types or diseases like miRNAs ???***

METHODS & RESULTS

We performed small RNA sequencing (NGS) to identify repertoire of piRNome in human diseases

(i) Prior to sequencing, we checked the expression of 4 Piwil in concerned tissues/cells

We observed expression of all four Piwil mRNAs from qPCR study except Piwil3.

(ii) Small RNA Sequencing was performed by *Genotypic Technology Pvt. Ltd*

Minimum of about 15-20 million reads per sample were generated from this small RNA sequencing project

(iii) Quality Check (QC) of reads: FastQC

(iv) Mapping of reads to human reference genome (hg19) using Bowtie

(v) Identification of known piRNAs: iMir and mirTools

(vi) Identification of novel piRNAs: After separating known piRNAs, the remaining unclassified reads are analyzed by piRNApredictor to identify novel piRNAs

(vii) Comparative expression analysis of piRNAs

- **A set of piRNAs were identified which could be used as biomarker for the disease**
- **piRNAs are mostly originated from the repeats & target the repeats**

Further studies on this problem are on-going as follows:

❖**Expression profiling analysis to identify differentially expressed mRNAs in this diseases using public databas or generating microarray data**

❖**Predicting targets of differentially expressed piRNAs (*sequence complementarity*)**

❖**Enrichment analysis of piRNA targets using MetaCore to identify biological processes or pathways predicted to be modulated by piRNAs**

❖ **Assessing the role of selected piRNAs by validations**