2016Joint Usage and Research Report

<table>
<tr><td colspan="2">Title of Research Project</td><td>Functional interpretation of the molecular interaction between long noncoding RNA and RNAi machinery in the nucleus</td></tr>
<tr><td rowspan="2">Applicant</td><td>Institution</td><td></td></tr>
<tr><td>Job title and name</td><td></td></tr>
<tr><td>Visiting researcher</td><td>Name</td><td>Professor Valerio ORLANDO
Head of KAUST Environmental Epigenetics Program
KAUST, Thuwal Saudi Arabia and Santa Lucia Foundation, Rome Italy</td></tr>
<tr><td colspan="2">Purpose of the Research Project
(approx. 250 words)</td><td>The key research topics of the collaboration are:

1. Role of nuclear RNAi components in gene expression regulation and chromatin architecture
2. Investigating the functional association of RNAi components with lncRNAs and their role in the assembly of nuclear bodies called paraspeckles
3. Understanding the role of lncRNAs (NEAT-1 and MALAT-1) in 3D genome organization</td></tr>
<tr><td colspan="2">Development of the Research Project and Results
(approx.. 850 words)</td><td>2. Research Project
The role of RNAi in post-transcriptional gene silencing in the cytoplasm is well characterized. Although, nuclear RNAi proteins (Argonaute-1 and Dicer) and long non-coding-RNAs (ncRNA) have shown to be enriched in the chromatin, however, little is known about their collective role in the cell nucleus and the mechanisms that regulate ncRNA function. The goal of this project is to investigate the nuclear functions of RNAi components and lncRNAs in mammalian cells, particularly in nuclear architecture dynamics in somatic cell response to environmental stress. A combination of genomics, protein complex biochemistry and imaging approaches are used.

Progress
To date, we have identified a novel role for nuclear Argonaute-1 in 3D genome organization and gene expression regulation in human cells. In this study, I discovered for the first time that nuclear Argonaute-1 associates with long-coding RNA and regulate 3D genome structure. To our knowledge this</td></tr>
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	is a breakthrough in the field of 3D genome biology and will further our understanding of the complexity of genome structure and function both in normal development and diseases. We presented this work in a Keystone symposium "Noncoding RNAs: From Disease to Targeted Therapeutics" at the Fairmont Banff Springs, Banff, Alberta, Canada. And a high impact journal manuscript " A novel role of nuclear Ago1 in regulating chromatin architecture and gene expression" Muhammad Shuaib, Krishna Mohan Parsi et al., 2017 is in preparation. Interestingly, we discovered another important function of nuclear RNAi components (Ago1 and Dicer1) in the assembly of NEAT-1-lncRNA containing nuclear bodies called paraspeckles. Paraspeckle nuclear bodies play important roles in various nuclear processes such as gene regulation and nuclear organization. A researcher from Orlando's lab, Dr. Muhammad Shuaib, visited Prof. Hirose's laboratory for three weeks (2016) to identify the functional association of RNAi components with NEAT-1- lncRNA and paraspeckle by using RNA-FISH. During this visit he successfully completed all the experiments and obtained very interesting results that complement our research project at KAUST. This work is under preparation for a second manuscript that we will publish soon in a well-reputed journal; "Chromatin associated RNAi components function in the assembly of NEAT1 lncRNA-containing paraspeckle. **Muhammad Shuaib** et al.," Moreover, we have identified a novel previously uncharacterized isoform of human Dicer that is highly enriched in the nucleus. we found a short unique peptide at the N-terminal of this protein, which we used for the generation of custom-made antibody. We have used this antibody for the characterization of this short isoform in various cell lines. The preliminary results indicate that this protein is present in different human cells, suggesting its essential role in the nucleus. Another collaborative project initiated with the group of Prof. Hirose is to investigate the function of long-non-coding RNAs (NEAT1 and MALAT1) in chromatin architecture by using high through-put chromatin conformation analysis (Hi-C). I used NEAT1, MALAT1 and double knockout cell lines that were obtained from the laboratory of Prof. Tetsuro, for the Hi-C experiments. We successfully completed the construction of all Hi-C libraries and obtained good quality data after sequencing in the KAUST core lab. The data is now available for further analysis and integration with other data sets, in order to complete the project. To further understand the mechanism and function of nuclear RNAi

components (Ago1 and Dicer1) we are using an integrated proteomic approach to decipher in vivo protein-protein interactions and applied this strategy to globally map the Ago1 and Dicer1 interaction network in human cells. This project involves the generation of stable cell lines expressing FLAG-HA-tagged RNAi components (Ago1 and Dicer1) proteins. By tandem affinity purification (TAP-TAG) and mass spectrometry analysis I have identified key components of paraspeckle nuclear bodies associated with Ago1 in chromatin fraction.

Furthermore, to study the nuclear localization and co-localization of RNAi components, we applied the genome-editing technology (CRISPR-CAS-9) for tagging of endogenous genes (Ago1 and Dicer1) with GFP. We generated stable HeLa cell lines expressing GFP-tagged endogenous Ago1.

Remaining Work and Expected Results

1) To complete the collaborative project with the group of Prof. Hirose, which involves investigating the function of long-non-coding RNAs (NEAT1 and MALAT1) in chromatin architecture. This project aims to explore a novel link between nuclear lncRNAs (NEAT1 and MALAT1) and chromatin associated RNAi components in regulating 3D genome structure. This work will lead to another high impact manuscript.

2) To fully understand the function of above mentioned newly identified short isoform of human Dicer, by CRISPR-mediated deletion, CHIP-seq and RIP-seq. The results from this work will clarify the nan-canonical role of nuclear Dicer and might open new avenue for future research.

Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	**Noncoding RNAs: From Disease to Targeted Therapeutics (J5)** February 5—9, 2017 Fairmont Banff Springs • Banff, Alberta Canada
	【Journals】