

Bankhead-Coley Cancer Research Program

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Project Title: KSHV-Encoded MicroRNAs

Project Summary: The goal of this research is to generate additional data in support of the team's research application to the National Cancer Institute titled "Kaposi's sarcoma-associated herpesvirus-encoded microRNAs." Kaposi's sarcoma-associated herpesvirus (KSHV), a virally-induced cancer, is the causative agent of Kaposi's sarcoma and lymphoproliferative diseases such as primary effusion lymphomas and a subset of Multicentric Castleman's disease. KS primarily afflicts the skin but in aggressive cases also affects internal organs including the lung, liver, and heart. Predominantly observed in patients with compromised immune systems, KS was originally identified in HIV-infected AIDS patients. In the absence of HIV, KS can occur in the context of organ transplantation and is currently rising in elderly populations. MicroRNAs (miRNAs), a new class of gene expression regulators, are small nonprotein-coding regulatory RNA molecules that bind to messenger RNAs and as a result decrease or stop protein production. Within the last 5 years, miRNAs have been identified in nearly all multi-cellular organisms. In humans, over 600 miRNA genes have been found, many of which are involved in regulating fundamental biological processes such as development, proliferation, and hematopoiesis. Recent reports have implicated aberrant miRNA expression patterns in many human cancers. Increased levels of miRNAs targeting tumor suppressor genes have been identified in breast, colon, lung, and prostate cancer. DNA tumor viruses encode miRNAs. In 2005, the team cloned miRNAs from KSHV positive lymphoma cells. At that time, 11 distinct miRNAs of 21 to 23 nucleotides in length were identified that perfectly align to the KSHV genome. Surprisingly, all candidate miRNAs mapped to a single genomic region known to be important for viral pathogenesis. Based on these findings, the team has hypothesized that viral miRNAs contribute to pathogenesis and tumorigenesis. To address this hypothesis, the original proposal aims to answer three questions:

1. When and where in the infected patient and in tumors are miRNAs expressed?
2. What cellular mRNAs do viral miRNAs target for degradation?
3. Are miRNAs required for the biology and pathogenesis of KSHV?

The proposal was received with enthusiasm and was felt to be highly significant to the field of cancer research; however, due to the extreme novelty of miRNA genes in tumor viruses, reviewers suggested providing proof-of-concept data that would further demonstrate the feasibility of the studies. In direct response to these reviews, the following three specific aims were established:

1. Establish robust miRNA reporter assays for KSHV-encoded miRNAs.
2. Complete detailed analysis of transcripts that express miRNAs during latent and lytic replication.
3. Engineer miRNA expressing cell lines to perform proof-of-concept gene expression profiling experiments

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In Aim 1, sensitive and specific reporter assays will be developed to detect miRNA expression for all KSHV miRNAs. The development of these assays, for which the team has provided ample preliminary data, will be important for Aims 2 and 3. Next, the above assays will be utilized to investigate under which conditions miRNAs are expressed in KSHV-infected lymphoma cells. These experiments will provide the basis for translational studies in which the team will probe for miRNA expression in tumor samples of patients suffering from various KSHV-related malignancies. These studies are in collaboration with clinical investigators at the University of Miami, the University of Florida, and the NCI, Frederick. Finally, we will express miRNAs in tissue culture cells and ask whether they induce changes in host cellular gene expression. For the gene expression profiling studies, the team is composed of experienced investigators including a bio-informatics specialist, who will be crucial for data mining and analysis. In summary, these experiments will generate the necessary analytical tools and preliminary data towards the identification of host cellular genes that are targeted by KSHV-encoded miRNAs.