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TITLE
PHENOTYPIC CHARACTERIZATION OF ENDOTHELIAL CELLS INFECTED WITH KSHV MICRORNA KNOCKOUT VIRUSES

HYPOTHESIS:
Since KSHV miRNAs are expressed during latency and latency is associated with malignancy, we hypothesized that miRNA knockout viruses would show impairment in producing the phenotypic changes associated with transformation of endothelial cells.

BACKGROUND/AIMS:
In Kaposi’s sarcoma (KS), endothelial cells which are latently infected with Kaposi’s sarcoma-associated herpesvirus (KSHV) become transformed. Precisely how this happens and which viral genes are responsible is still not clear. Among the limited set of genes expressed during latency are the twelve microRNAs (miRNAs). Recently, our laboratory has generated a complete set of KSHV miRNA knockout mutants, with each mutant virus lacking one of the twelve miRNAs. In addition, we have created a mutant lacking the miRNA cluster (miR-K12-1 - miR-K12-9 and miR-K12-11) and one lacking all miRNAs.

METHODS:
We infected Telomerase-Immortalized Vein Endothelial (TIVE) cells with each of the mutant viruses and examined the resultant phenotypes. Wild type- and mutant-infected as well as uninfected cells were evaluated by their growth rate, migration speed, ability to form tubules in Matrigel, propensity to undergo aerobic glycolysis, and expression of various blood and lymphatic endothelial cell markers.

RESULTS & CONCLUSIONS
It was found that there was broad variation between the different viruses in these aspects. For example, cells infected with delta-miR-K12-11 were found to have a reduced rate of migration when compared to wild type-infected cells. At the same time, cells infected with the delta-miR-K12-10 virus showed dysregulated metabolism. There were differences in the growth rate of the different cell lines as well, with delta-miR-K12-3, -8, and -11 showing a significant impairment. Taken together, the results demonstrate that KSHV miRNAs play different roles in inducing the phenotypic changes which are characteristic of transformed cells.