Investigation of the role of deregulated miR-654 and miR-4454 in melanoma pathogenesis and progress.

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The incidence of melanoma has continually increased mortality rates over the past decade in the United States. In 2013, it was estimated that 76,690 individuals (both male and female) were diagnosed with new cases of cutaneous melanoma, and out of those cases, 9,480 resulted in death. MicroRNA(miRNA)s are endogenous, 22 nucleotide non-coding small RNAs, which can regulate gene expression in animals and plants by complementary base-pairing to the mRNAs of target genes- which specifies mRNA cleavage or translation repression. We have established a distinct set of miRNAs associated with invasive and aggressive melanoma phenotypes and investigated their role in the invasion and migration of malignant melanoma cell lines Sk-Mel-26 and A375P. Recently Liu et al. 2022 reported a tumor-suppressing function for miR654-5p in colorectal cancer. This microRNA was one of the highlighted microRNAs in our previous study. We showed in the previous studies that miR-4454 acts as a tumor suppressor in A375P cells using invasion and migration of assay which we repeated the assay with Sk-Mel-26 cell line too. After seeding the cells and transfecting them with miR-654 or miR-4454, a control scrambled sequence, and miR-654 or miR-4454 inhibitor (upon reaching 60%-70% confluency, cells were well subjected to a scratch and were imaged at different time points. Image J (NIH website) was used to measure the area between the edges of the scratched monolayer from at least three locations per well at different time points. These results were compared against a control cell, transfected with a scrambled sequence but did not generate any miRNA. After transfecting A-375P and Sk-Mel-26 cells with miR-4454 the migration rate significantly increased (P-value= 0.01 and P-value= 0.018 accordingly) after 48h post scratch. The migration of transfected cells with miR-654 significantly decreased after 24h and 48h (p-value=0.00019). Thus, miR-4454 acts as an oncomir and miR-654 can be considered a tumor suppressor where their upregulation is associated with a decrease in melanoma cell proliferation and migration. Our work with inhibitors (specifically miR-654inhibitor) did not produce predicted results (no significant reversing effect) which might be due to the endogenous level of the microRNA and it will need future investigation using real-time RT-PCR.