

Identification of microRNAs regulating expression of vimentin gene and location of miR-17-3p targets beyond vimentin

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Introduction

MicroRNAs (miRNAs) are a group of small non-coding RNA sequences that are approximately 22 nucleotides in length. They play a significant role in regulating gene expression by binding to protein-coding regions of mRNAs, thereby repressing translation or degrading the mRNA altogether [1].

MiRNAs affect a variety of key cellular processes, such as cellular differentiation [1] and proliferation [2]. Furthermore, aberrant miRNA levels have been noted in a number of diseases, including several types of cancer, where miRNAs have demonstrated the ability to act as tumor suppressors and oncogenes [3].

Presently, there are only five miRNAs linked with prostate cancer, three as oncomirs and two as tumor suppressors. Prostate cancer is the second leading cause of cancer deaths for men in the United States and the most frequently diagnosed malignant tumor [4]. Studies have indicated that levels of vimentin, an intermediate filament with a key role in providing structural support for organelles in the cytosol [5], are correlated with prostate tumor growth [6]. Additionally, vimentin affects cell motility, a contributing factor to the development of metastatic phenotypes. One study comparing three genetically related sublines demonstrated higher vimentin levels in the metastatic and highly tumorigenic cell line than in both the slightly tumorigenic and non-metastatic cell lines [6].

A number of interactions between miRNAs and their target mRNA regions have been identified, but the mechanisms behind many miRNA target recognitions have yet to be determined. An investigation into possible miRNAs affecting vimentin expression yielded a correlation with miR-17-3p, a member of the miR-17-92 cluster, which was shown to bind to the 3'-UTR of the vimentin mRNA. Laser Capture Microdissection (LCM) analysis of RNA extracted from human prostate tumor samples confirmed the

lack of miR-17-3p in normal glandular epithelium or stroma, indicating the role of miR-17-3p as a tumor suppressor. Unlike some of the other members of the miR-17-92 cluster, miR-17-3p has yet to be investigated in depth [7].

As the role of miR-17-3p appears to be significant, one of the goals of the current investigation is to identify other targets of miR-17-3p in hopes of elucidating the repertoire of mRNAs regulated by miR-17-3p. In addition, although most studies in the past have focused primarily on the 3'-UTR of potential targets, a few investigations into the coding regions and 5'-UTRs of mRNAs have been done. It is now proposed that miRNAs may bind to these regions as well as 3'-UTRs [8]. Therefore, the 5'-UTRs and coding regions will also be taken into consideration in evaluating miR-17-3p target candidates. This study also aims to locate other miRNAs that affect the expression of vimentin.

There are three types of miRNA targets, depending on the degree of base pairing between the miRNA and the mRNA target sequence and the location on the miRNA at which the highest number of base pairings occur. Most miRNAs bind to their targets through sequences of around seven nucleotides located either one or two nucleotides from their 5' ends, known as seed regions [7, 9].

Many databases have been developed to produce potential targets for a given miRNA sequence, with each database possessing a distinct set of rules regarding the validity of a target. For example, TargetScanS requires complete complementarity between the targeted mRNA and the seed region, while PicTar allows for a certain number of mismatches with the seed sequence as long as a specific binding-energy threshold is met [9].

Methods

The most common means of detecting miRNA targets is to use bioinformatic tools to predict potential candidates and then implement laboratory techniques to investigate the actual roles of the predictions [4]. The current study aims to follow this approach by implementing a Perl program designed to detect the number of base pairings between the vimentin gene and all miRNA sequences. If the number of pairings meets/exceeds a certain threshold, the miRNA will be a potential candidate for laboratory testing. Proven interactions between miRNA and mRNA will be analyzed to determine

the average degree of complementarity between the bases, which will dictate the threshold value to be used in the Perl program.

After the candidates are identified, testing will be done through several experimental approaches in the Zehner Lab to determine if miR-17-3p is in fact binding to the predicted targets. First, the target protein region will be fused to the luciferase reporter gene. Luciferase activity will be observed after transfection into both M12 cells lacking miR-17-3p and M12 cells with a stable over-expression of miR-17-3p. The results should indicate a reduction in reporter gene activity in the presence of miR-17-3p. To confirm that the results are due to the binding of miR-17-3p, a mutation will be made in either the target sequence or the seed region of the miRNA. If miR-17-3p binding is in fact the cause of reduction in gene expression, there should be no difference in luciferase activity between the M12 and M12+miR-17-3p cells. Second, a Western blot will be used to measure and compare endogenous expression of the predicted targets in M12 and M12+miR-17-3p, depending on antibody availability. It is expected that protein levels should decrease in M12+miR-17-3p cells. Third, q-RT-PCR will be used to quantify mRNA levels for the specific protein targets and to determine whether miR-17-3p is interfering through mRNA degradation or by repressing translation. The results will be compared to proteomic analyses of human prostate tumors.

Implications

Currently, androgen ablation therapy is the most effective method for treating disseminated diseases, as its function is to prevent the growth of prostate cells [4]. However, androgen ablation is not successful, as prostate cells eventually develop into androgen-independent (AI) cells, bypassing the need for hormones and continuing to grow. Thus, it is worth identifying any miRNAs with significant functions in prostate tumor growth, as well as the corresponding targets, so that more effective treatments can be established.

As miRNA expressions have been demonstrated to vary between types of cancer and between normal and affected individuals, miRNAs have the potential to serve as powerful biomarkers in the future for diagnostic and prognostic purposes [10]. This study will hopefully shed more light on the function of the vimentin gene in prostate

cancer and provide further information regarding the role of miR-17-3p in the human genome.

References

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