SUMMARY OF THE WORK DONE

Scope of the work

One of the major breakthroughs of the century in Biological science is the discovery of RNA interference (RNAi), a mechanism that regulates gene expression at the post-transcriptional level in the living cells. This phenomenon was initially described by other generic names, such as co-suppression and post-transcriptional gene silencing (PTGS). RNAi is an RNA-dependent gene silencing process that is controlled by the RNA-induced silencing complex (RISC). Though major studies on RNAi have been focused in the cytoplasm, many factors are also active in the nucleus. One of the best examples is AGO2, a cytoplasmic protein that regulates transcription in humans.

Mainly, two types of small RNA molecules – the miRNA and siRNA are central to RNA interference. In eukaryotes, RNAi pathway is initiated by the enzyme Dicer, which cleaves long double-strandedRNA molecules into short double-stranded fragments of ~20 nucleotide siRNAs. Each siRNA is unwound into two single-stranded RNAs (ssRNAs)- namely, the passenger strand and the guide strand. The passenger strand undergoes degradation while the guide strand gets incorporated into the RNA-induced silencing complex. Some RNA-binding proteins TRBP and PACT are known to contribute to this process, their mode of binding to Dicer and their genome-wide effects on small RNA processing have not been determined. Here, we have identified that the oncogenic protein, Glioma amplified sequence-41(GAS41) is required for the effective RNAi processing. We have also demonstrated that GAS41 and other RNAi factors are present both in nucleus and cytoplasm and potentially interact with both nuclear and cytoplasmic factors. This delineate that GAS41 is a member of two separate complexes having two distinct functional roles. Impairment of GAS41 by siRNA resulted in modulation of a different subset of miRNA in nuclear compartment and cytoplasmic compartment. Overall this study describes the essentiality of GAS41 in RNAi biogenesis and brings new insight in the field of gene regulation.

miRNAs are 21-23 nucleotide single-stranded RNA molecules that are found in eukaryotic cells. They exert their activity by binding to the 3-prime UTR of the

cognate gene. They are involved in diverse biological activities such as tumorigenesis, immune response, insulin secretion, neurotransmitter synthesis and circadian rhythm. They may play a direct role by interacting with the gene promoter or may be involved in regulating gene expression via epigenetic regulation such as DNA methylation and other histone modification. Several studies have substantiated the role of miRNAs in cancer progression. They may either act as oncomiR by supporting tumor growth or may act as tumor suppressor miRNA.

In addition to the miRNAs and siRNAs, several other types of small RNAs have been discovered like the Piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNAs) and the repeat associated small interfering RNA (rasiRNA), which execute functions of RNA silencing under different conditions and stages.

Glioblastoma is a serious life-threatening brain tumor in humans. A cohort of aberrantly expressed miRNA and genes lead to aggressive invasive properties. In our study, we have shown for the first time that tumor suppressor miR-203 which is highly downregulated in glioblastoma have a significant connective role with GAS41. By suppressing GAS41 expression, the proliferation of glioblastoma was effectively controlled by up-regulating the expression of a tumor suppressor miRNA 203.

Statement of problem

The aim of this study is to understand how Glioma Amplified sequence-41(GAS41) interconnects RNAi factors and cancer via small miRNA. Two studies are focused extensively on addressing this problem. In the first part of the study, we have identified the interacting partners of GAS41 by mass spectrometry analysis by overexpressing GAS41 and subsequently performing immunoprecipitation studies using anti-flag beads. Confirmation of the interaction was performed by coimmunoprecipitation, GST pull down and reciprocal coimmunoprecipitation studies.

Nuclear and cytoplasmic localization of GAS41 were isolated by cellular fractionation, coimmunoprecipitation, and ammonium sulfate precipitation. miRNA profiling study was performed in human glioblastoma cells to identify the role of GAS41 in miRNA processing in human glioblastoma . The study unveiled GAS41 as a novel target for endogenous miR-203 and also demonstrated an inverse correlation

of miR-203 expression with GAS41 in glioma cell lines (HNGC2 and U87). Overexpression of miR-203 negatively regulates GAS41 expression in U87 and HNGC2 cell lines. Moreover, miR-203 restrains miR-10b action by suppressing GAS41. GAS41 is essential for repressing p53 in tumor suppressor pathway during cell proliferation. Enforced expression of GAS41 produced a contradictory effect on miR-203 but was able to enhance p53 tumor suppressor pathway-associated protein. It was also found that miR-203 maintains the stability of p53 as knockdown of p53 expression using siRNA resulted in downregulation of the expression of pri-miR and mature miR-203. Conversely, reconstitution of miR-203 expression induced apoptosis and inhibited migratory property of glioma cells. Taken together, we show that miR-203 is a key negative regulator of GAS41 and acts as tumor suppressor microRNA in glioma.

Objective of the Study

1. To identify the role of Gas 41 in miRNA biogenesis.

2. To identify the role of miR-203 in inhibiting human glioblastoma proliferation and migration by suppressing GAS41.

Methodology Used and Sample Results

GAS41 is a well known conserved protein. It is frequently amplified in several cancers especially in brain tumor. A recent study in *Drosophila* showed GAS41 as a component of the RNAi pathway that interact with Dicer. In addition, our study also proved that GAS41 is an integral component in mammalian miRNA pathway and promotes miRNA biogenesis. We have identified by mass spectrometry analysis that GAS41 forms stable complex with the miRNA components. This observation was further verified by coimmunoprecipitation and reciprocal coimmunoprecipitation. Immunodepletion assay also strongly supports our earlier hypothesis that GAS41 is indeed a component of the miRNA biogenesis. Immunoprecipitation assay also suggests that GAS41 interacts with endogenous components of the miRNA pathway. Furthermore, we have examined the type of interaction that exists between GAS41 and RNAi. For this, we have treated the immunoprecipitated product with RNAase which showed that the interaction is merely a protein-protein interactio. We have hown that GAS41 is distributed both in nucleus and cytoplasm and interacts with both

cytoplasmic and nuclear miRNA components. Ammonium sulfate precipitation revealed that GAS41 form two different complexes in cytoplasm and nucleus. Global miRNA survey data also deciphered that impairment of GAS41 leads to alteration in the expression of a few subset of miRNA. This was further validated by quantitative Real-time PCR. Further results of RNA immunoprecipitation showed that let-7b5p specifically interact with GAS41, which strongly suggest that GAS41 is indeed required for the regulation of some miRNA.

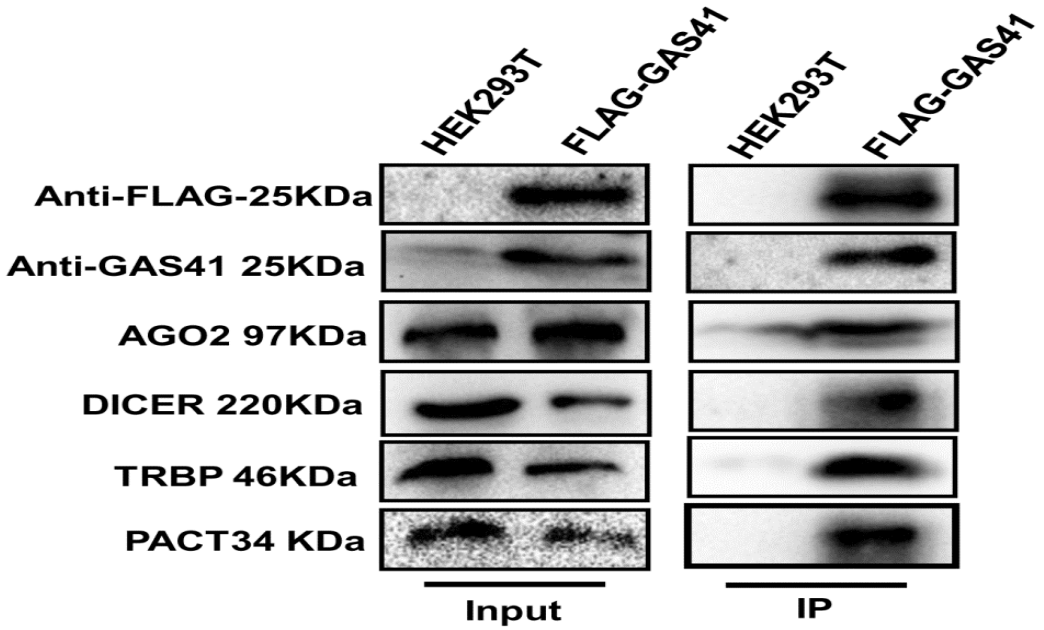

Figure 1: Result showing GAS41 interact with RNAi component for objective-I

Gliomas are the most frequently occurring neuroepithelial brain cancer arising from glial cells in the brain. It accounts for 12–15% of all brain tumor and are categorized into four grades (I–IV) according to the World Health Organization (WHO). Among all glioma cases diagnosed, astrocytoma grade III or glioblastoma multiform (GBM) is considered to be the most severe and incurable form due to poor prognosis and high invasiveness. Therefore, further research in understanding the regulatory mechanism that discloses the molecular mechanisms of pathogenesis of glioblastoma is of utmost need. MicroRNAs are involved in several cellular processes and their misexpression is often seen in various tumors. Several studies have reported misregulation of miRNA in glioblastoma, but no relation between miRNA and GAS41 in glioblastoma been reported till date. In this report, we have shown that GAS41 acts as a novel target

for miR-203 where an inverse correlation exists both transcriptionally as well as translationally. Over-expression of miR-203 resulted in downregulation of GAS41 that lead to induction of p53/p21/p14 tumor suppressor pathway. Interestingly, miR-203 also downregulates miR-10b expression by repressing GAS41 in gliomas, causing a proficient induction of apoptosis and preventing migration. Altogether this study put forward a new role of miR-203 as a tumor suppressor miRNA that in turn control GAS41 expression in human glioblastoma cell-line.

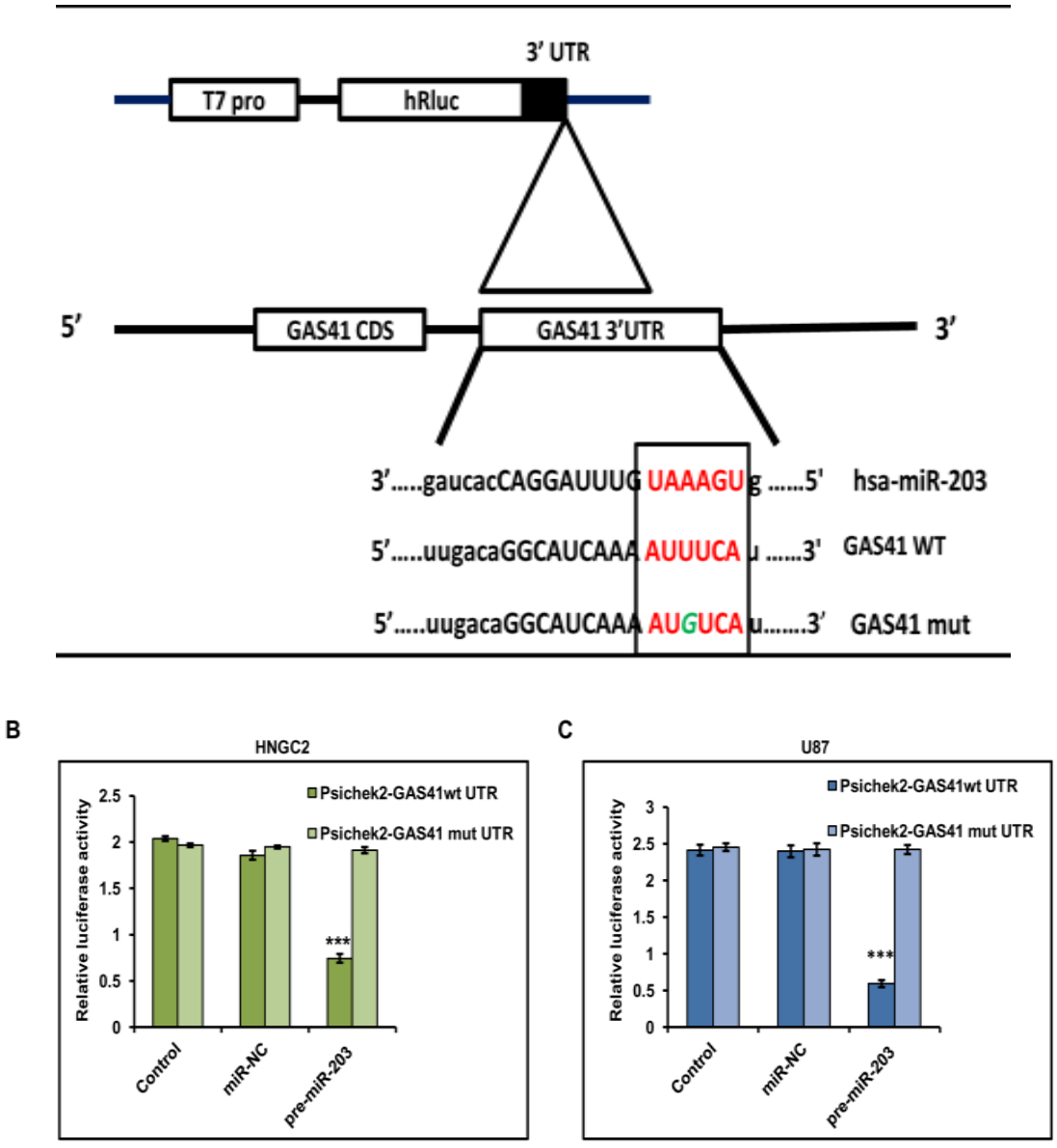

Figure 2: Graphical representation shoes GAS41 as a target of miR-203 and validation by luciferase assay. Objective-2