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In Silico Characterization of Hras Pathway for Therapeutic Implications Against Cancer

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ABSTRACT

Hras (v-Ha-ras Harvey rat sarcoma viral oncogene homolog) plays a pivotal role in breast tumorigenesis. Expressional aberrations in Hras has significantly been correlated with patient's poor overall survival. In the present study, in silico characterization of Hras to trigger downstream effectors including raf, mek, erk has been explored. Three mutational hotspots for Hras (codons12, 13, 61) have been retrieved from the literature. Promoter modelling using Proscan software revealed a promoter region lacking TATA sequence. To study the epigenetic modifications, methylation analysis of promoter lacking TATA box was done using Methylator software. Methylation is directly correlated with tumor targeting therapies as it represses expression of certain oncogenes. Five candidate sites for hypermethylation of Hras were predicted. Analysis of protein residues involved in interlocking with its downstream effector proteins was also uncovered. In addition to this, a diagnostic and therapeutic relevance of micro RNAs (miRNAs/miRs) specifically acting either as oncogene or tumor suppressors on Hras mediated pathway have been identified. Seven micro RNAs (Let-7, miR-195, miR-497, miR- 184, miR-34a, miR-34c, miR-155) found to be responsible for down regulating expression of proteins involved in rassignalling cascade while miR-372 and miR373, miR-212, miR-206/21 and miR-17-92 cluster are known to elevate rassignalling pathway. Interestingly, dysregulated expression of Let-7, miR-195, miR-497, miR-184, miR-34, miR-155 co-related with poor prognosis in different cancers has also supported these in silico findings.

1. Introduction

Cancer is one of the leading causes of deaths worldwide. According to a recent report [1], breast cancer accounts for the maximum number of female deaths. To surmount cancer associated impediments, scientists and physicians globally are striving hard to formulate advance methodologies for early detection and treatment of cancer. Also because of the challenges that have been presented by the conventional practices in oncology research, conception of potent approaches for disease diagnosis and therapeutics has become need of the hour. Rasoncogene family is involved in cell growth signal transduction pathway. So far three commonly mutated ras genes detected in human carcinomas are Hras, Kras and Nras [2]. Over expression of Hras significantly regulates conversion of normal cells to cancerous form as observed in breast, gastric, urinary, head and neck cancers [3, 4]. Early detection and treatment of breast cancer using micro RNAs (miRNAs/miRs) has also been established. These non-coding RNA molecules (20-25 nucleotides) are responsible for regulating vital cellular processes including apoptosis, persistent proliferation, invasion and metastasis [5]. Recently their involvement in tumorigenesis has been identified because of their paramount role in modulating cancer related activities and their location near cancer associated regions in genome [6]. Additional studies have reported a strong relationship between aberration expression of miRNAs and oncogenesis [7]. In this study, in silico characterization of Hras has been done using different bioinformatic approaches from gene to protein levels. In addition to this, a set of miRNAs involved in Hras mediated pathway (including Raf, ERK molecules) has been identified. Expression of these identified oncogene and tumor suppressor miRNAs subsets were further validated by published data. Tumor suppressor miRs were aligned with their respective mRNA targets to determine sites where binding takes place.

2. Methodology

2.1 Data Mining and Curation

Initial task was to extract relevant information about Hras, its DNA and protein sequences, isoforms expression dysregulation in human. Data mining was done in order to extract the relevant information from scientific databases and journals such as PUBMED, Science Direct, Oncogene, BioMed Central and Oxford journals. OMIM (Online Mendelian Inheritance in Mammals) has been consulted for its discovery and gene localization reports.

2.2 Promoter and Regulatory Sites Prediction

Promoter was predicted by providing it with 2242

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base pair upstream region in silico using proscan promoter prediction tool [8, 9]. Analysis of epigenetic modifications such asmethylation pattern over the promoter region was done using reported bioinformatic tool, Methylator [10]. Tool used for finding and analyzing CpG islands in Hras gene was EMBOSS CpGplot [11]. Also, literature search lead us to identify mutational hotspots in Hras gene.

2.3 Protein structure

Primary protein structure of Hras protein was retrieved from National Center for Biotechnology Information (NCBI). Its secondary structure was predicted using bioinformatics tools. 189 amino acid long protein sequence of Hras was retrieved from NCBI. Chau Fasman secondary structure predictor and next prot protein navigator was used for secondary structure prediction of the given sequence. Quaternary structure of Hras gene was also restored from PDB (Protein data bank) and was labelled after.

2.4 Molecular Interacting with Hras

Molecular cross talks of Hras with downstream elements Raf proto-oncogene serine/threonineprotein kinase, which is the part of protein kinase cascade has been studied using Kegg pathway database. Extensive literature search was done to interpret the conceived information.

2.5 Identification and Characterization of miRNAs in Hras Pathway

A panel of miRNAs responsible for down regulation of proteins involved in rassignalling pathway was found out by mining literature. Once found, miRNAs were aligned with their target mRNAs using RNA22 software, a bioinformatic tool used to find complementary region between sequences. Sequences of miRNAs were taken frommi RNA database and that of mRNAs were taken from ensemble.

3. Results

3.1 Structural and Functional Characterization of Hras

Hras gene had ensemble Id ENSG00000174775, the gene was found to be oriented on minus strand. It codes for 2 isoforms and the coding positions range from 533979 to 537287. Somatic and genetic mutations in Hras were studied showing mutational hotspots at codon 12, 13 and 61 [12]. Mutations at these positions can be pernicious. Protein structure was studied at all levels. Secondary protein structure was investigated and was found to have a total 73% of helixes, 41% beta sheets and 13.2% turns. Labelled quaternary protein structure is also shown in the Fig. 1.

3.2 Epigenetic Modifications

In depth analysis of Hras gene lead to the study of epigenetic modifications. Five hypermethylation sites over promoter were uncovered.

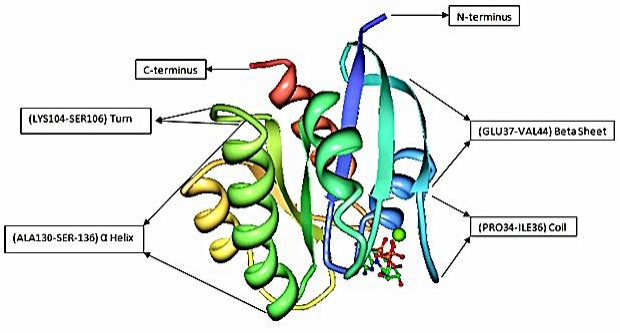

Fig. 1: Labelled structure of Hras protein. Structure showing C, N terminus with alpha, beta sheets and turns.

3.3 Micro RNA Characterization and Alignment

Different miRNAs are involved in the regulation of MAPK pathway. These were characterized according to their oncogenic or tumor suppressor behavior in rassignalling (Table 1). Micro RNAs responsible for down regulating the hyperactivity of ras family members were aligned with their respective targets to figure out exact target sites that can be subjected to further research for site directed mutagenesis (Fig. 2).

Table 1: Oncomirs and tumor suppressor miRNAs along their targets

Target	Oncomirs	TS miRNAs
Ras	miR-372,miR-373	miR-204/ let-7
Raf	miR-212	miR-132/497
Mek/Erk	miR-206/21	miR-34a
C-myc	miR-17-92	miR-184
Srf		miR-133/miR-145
C-fos		miR-155

4. Discussion

Hras activation triggers downstream effector protein raf kinase, which phosphorylates threonine/tyrosine recognition kinaseMEK. Phosphorylated MEK activates mitogen activated protein kinase (MAPK) leading to increase release of transcription factor c-myc and c-fos. Inactivating any of the proteins involved in this pathway, can halt cell proliferation [13]. Studying epigenetic modifications of a gene can help in studying its expression. Hypermethylation at specific sites can aid in repressing oncogenes [14]. Predicted methylation sits at promoter region of Hras can play the desired role in reducing hyperactivity of Hras (Fig. 3). Micro RNAs are post transcriptional regulators. They repress target gene expression by bonding to complementary region in mRNA transcripts. Alignment of miRNAs with their target mRNA aided in finding the hotspots where mutation directed research can be done. RNA22 was the

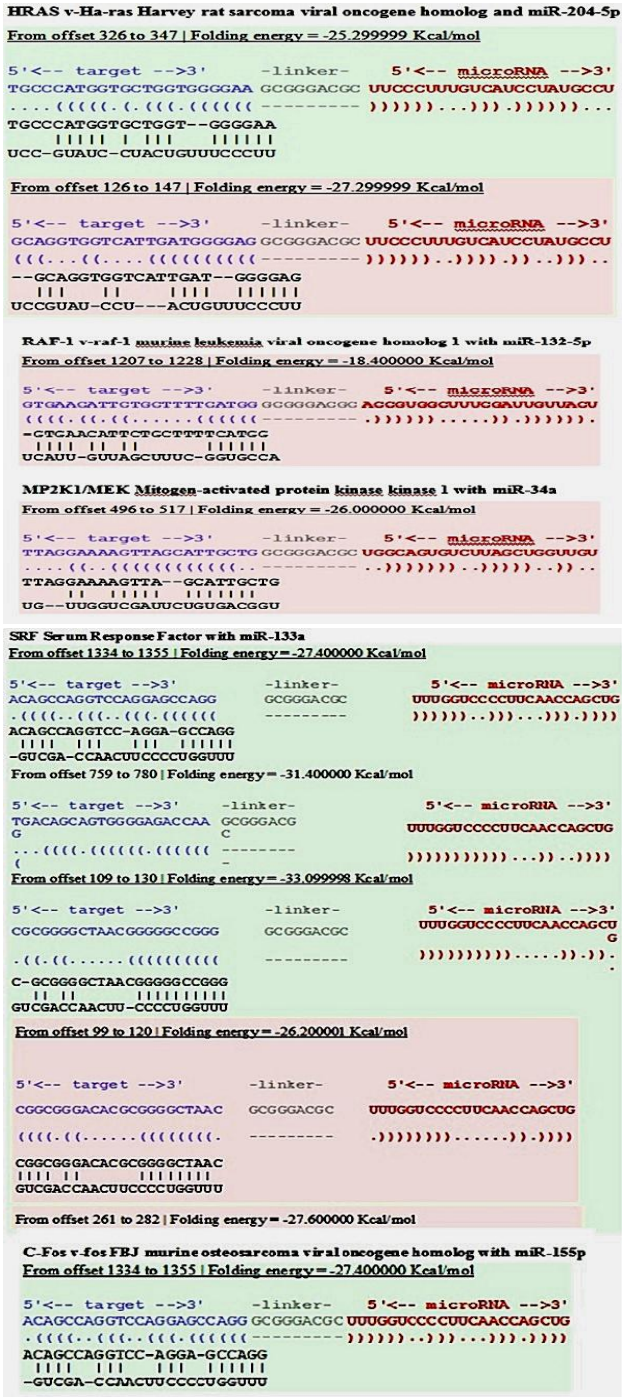

Fig. 2: Alignment of TS miRs with their respective mRNA targets

software of choice that aligned miRNA with their target mRNAs. Many miRNA sare reported in literature which are involved in regulation of key proteins of ras signaling pathway.

Members of miR-372 and miR-373 cluster have been found to elevate the expression of ras [15]. miR-212 increases Raf levels and miR-17-92 cluster works in accordance with c-myc in anti apoptotic mechanisms and

Fig. 3: Predicted Methylation sites at Hras promoter. Methylated cytosines of CpG dinucleotides are colored red. Hypermethylation at these sites causes repression of this gene

increases cell growth [16] whereas, miR-206/21 cooperate in hyper expression of Ras-Erk pathway [17]. Micro RNAs of let-7 family are known for down regulation of ras [18], miR-195 and miR-497 target raf and repress its expression [19], miR-184 acts as a break for c-myc [20] miR 34-a and miR 34-c are known to inhibit MEK and myc expression respectively [21]. miR-155 is known to repress transcription by repressing c-fos expression [22, 23] These microRNAs were mapped against their targets using RNA22 software to figure out hotspots for site directed mutagenesis (Fig. 4). Micro RNAs have vital role in examining prognosis of cancers as well. Diminished levels of miRNAs of Let-7 family have reported to be related to lymph node metastasis and poor prognosis in breast cancer [24]. Another report is cited for reduced levels of let-7 being related to poor survival in lung cancer patients [25]. MiR-195 has been identified as a marker for poor prognosis in adenocortical cancer [26]. Low levels of miR-497 distinguish between short term and long term survival in pancreatic cancer patients [27]. High levels of miR-184 were reported in early and advance stage squamous cell carcinoma (SCC) [28, 29]. MiRNAs of miR-34 family serve as one of the strong prognostic markers in non-small cell lung cancers.

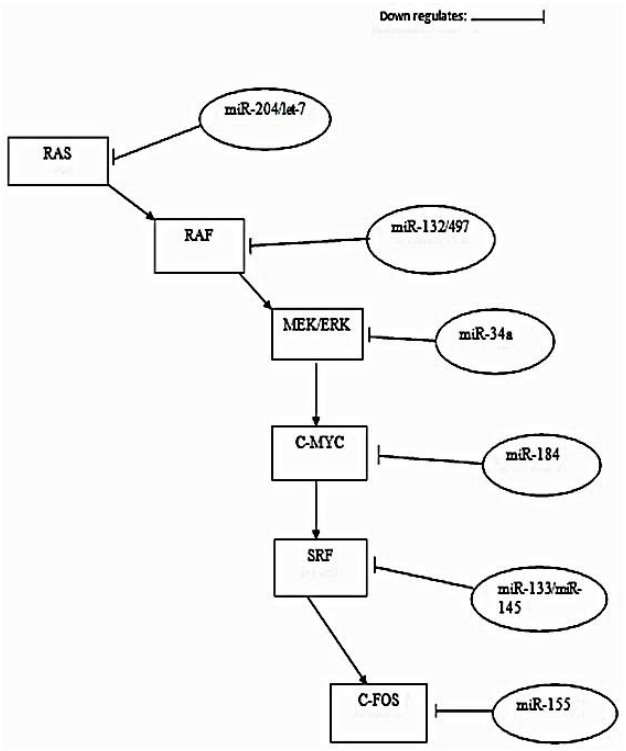

Fig. 4: Micro RNAs involved in down regulation of RAS pathway

Reduced levels of miR-34a distinguished between normal and cancerous lungs and also indicated high probability of disease re-occurrence [30]. An abberated expression of miR-155 is reported in several types of lymphomas. Deregulated expression of miR-155 was related to various bio pathological features of breast cancer [31].

5. Conclusion

Repression through miRNAs is one of the very important regulatory mechanisms. Using the set of miRNAs described above can help achieving novel methodologies in breast cancer diagnosis, prognosis and therapeutics. Using oncogenic miRs as a target for repression can innovate new therapeutics in breast cancer research. Similarly over expressing set of tumor suppressor miRs can help repress tumorgenicity. Hypermethylation at promoter region can downturn Hras expression as well altering residues responsible for accurate protein-protein interaction of Hras with raf can hamper ras signaling pathway and can contribute significantly in anticancerous therapeutics.

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