An Exploration of the GRB2 Gene

Gene Topology

The location of the GRB2 gene was initially characterized in 1994 by two studies – one by Huebner et al. and another by Yulug et al.. Huebner et al. mapped it by probing various chromosomal fragments with cDNA for the GRB2 gene and identifying hybrids using Southern blot (Huebner et al., 1994). They found that the GRB2 gene was located somewhere from 17q22 to the 17q terminus. Yulug et al. were able to map the gene more accurately using fluorescence in situ hybridization to 17q24-q25 (1994). Thanks to modern sequencing approaches, we know that GRB2 is located precisely on chromosome 17 on the complement strand at 17q25.1 (NCBI, GeneID: 2885).

Transcription of GRB2

GRB2 is a signal transduction protein that is universally expressed (Fagerberg et al., 2014). The GRB2 gene has 6 exons but only 5 exons are utilized in the primary isoform. The secondary isoform contains 3 exons in the mature mRNA (NCBI, GeneID: 2885). There are nine promoters listed by GeneCards, but only 4 of them have strong evidence backing them so those four will be assumed to be true promoters (Fishilevich et al., 2017; Safran et al., 2022; Stelzer et al., 2016). In addition to those promoters there are 36 enhancer loci in various upstream and downstream locations but only 13 of them are strongly supported and will be assumed to be true enhancers. The two factors with the highest binding site affinity for the GRB2 promoters and enhancers are p53 and Sp1 but there are around 200 transcription factors that are listed for the binding sites (Fishilevich et al., 2017; Safran et al., 2022; Stelzer et al., 2016). Both Sp1 and p53 act as pioneer factors, meaning they are the first factors to bind and recruit other transcription factors, but p53 may also act as an inhibitor to Sp1 by binding to it (O'Connor et al., 2016; Sullivan et al., 2018).

GRB2 Translation

The GRB2 protein undergoes translation via the canonical eukaryotic ribosomal pathway, but the protein regularly undergoes lysine acetylation and threonine phosphorylation post-transcriptionally (The UniProt Consortium, 2021).

Expression Regulation

Gene expression can be modified on a few fronts: chromatin modification, transcriptional control, mRNA interference, and post-translational modification. The aforementioned transcription factor Sp1 is a good example of the first two because it binds the GRB2 promoter and recruits other transcription factors, but it also recruits lysine acetyl transferases which acetylate histones to open up the chromatin and give transcriptional machinery access to the DNA (O'Connor et al., 2016; Clark et. al., 2019, Ch. 17). The GRB2 gene also has a repressing mechanism controlling its mRNA in the form of a microRNA named miR-27b-3p (Zhang et al., 2021). This miRNA binds to the 3' untranslated region of the GRB2 mRNA and causes it to be degraded. One example of a pathogenic post-translational modification is the deubiquitination of GRB2 by a deubiquitinase named PSMD14 (Lv et al., 2020). PSMD14 is overexpressed in liver

cancer and prevents the GRB2 protein from decaying, promoting tumorigenic signaling in the RAS/ERK pathway (Lv et al., 2020; Giubellino, 2008; Molina & Adjei, 2006). This implies that degradation of GRB2 is signaled by ubiquitination as well.

Other RNAs Involved

As previously mentioned, miR-27b-3p binds to the 3' untranslated region of the GRB2 mRNA and causes it to be degraded (Zhang et al., 2021). In addition, there are four lncRNA and a miRNA encoded in the intronic regions of the gene (NCBI, GeneID: 2885). The functions of these RNAs are not known, but they must contribute to a secondary structure of the mRNA by the very nature of miRNA synthesis requiring hairpin formation.

GRB2 mRNA Interference by miR-27b-3p

In a study by Zhang et al. the authors sought to establish the role of the transcription factor PAX4 in gastric cancer and discovered that it upregulates GRB2 production by downregulating expression of a GRB2 repressor, the microRNA miR-27b-3p (Zhang et al., 2021). For the purposes of this paper the analysis will be focused on the portion of their study where they determined that miR-27b-3p binds to the 3' untranslated region of the GRB2 mRNA.

After establishing that elevated PAX4 levels contributed to malignant cell growth, they used bioinformatic tools to find targets for PAX4. They discovered 3 binding sites for PAX4 in the miR-27b-3p promoter and after validating PAX4 binding, they discovered that PAX4 and miR-27b-3p expression are negatively correlated. It was further established that miR-27b-3p acted against the effects of PAX4, promoting apoptosis, limiting cell motility, and limiting cell growth. The next logical step was to look for targets of miR-27b-3p, and this is how GRB2 came into focus.

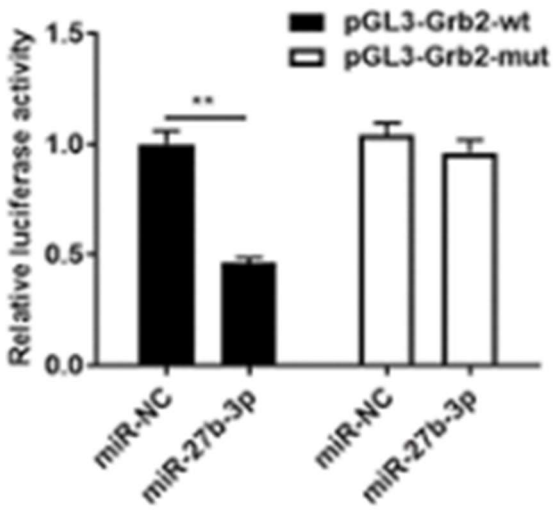

Figure 1: Dual luciferase assay results

Using bioinformatics tools they discovered that miR-27b-3p potentially targets the 3' untranslated region of GRB2 mRNA and this is where the more detailed discussion starts. To confirm the bioinformatic information they used a gastric cancer cell line which was transfected with miR-27b-3p mimics and a negative control gastric cancer cell line to perform a dual luciferase assay. The luciferase reporter plasmids either had a wild-type 3' region or a mutated 3' region to see how miR-27b-3p regulated the transcripts. They found that miR-27b-3p was able to reduce the luminance from the wild-type plasmid but not the mutated plasmid. This agrees with the bioinformatic data and confirms that miR-27b-3p binds to the 3' untranslated region of the GRB2 mRNA transcript.

Next, they decided to quantify GRB2 expression in gastric cancer patient tissue samples and in gastric cancer cell lines. They use qRT-PCR to test each sample and line and found that GRB2 was significantly overexpressed in all cancer tissue samples and cell lines compared to normal, patient matched tissue, and comparable healthy gastric mucosal cells.

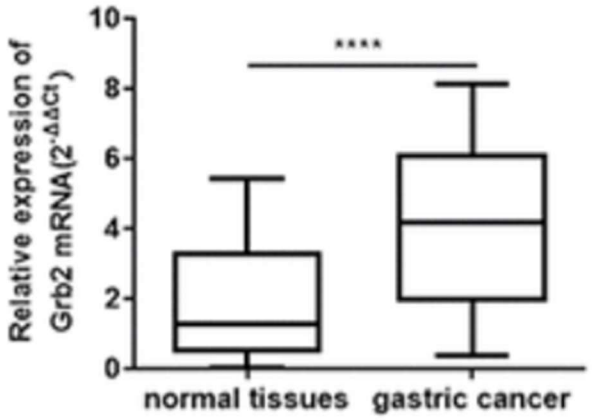

Figure 2: Patient sample GRB2 expression

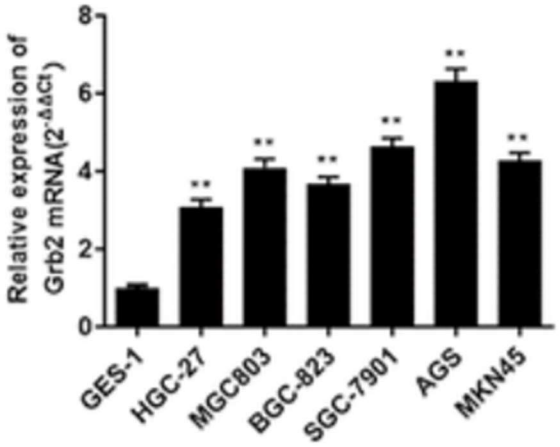

Figure 3: Cancer cell line GRB2 expression compared to normal cell line GES-1

In addition to these untreated cell lines, they also assayed their transfected cells. They found that cells transfected with miR-27b-3p mimics express significantly less GRB2 when compared to their negative control, and cells transfected with miR-27b-3p inhibitors showed higher GRB2 expression than their negative control.

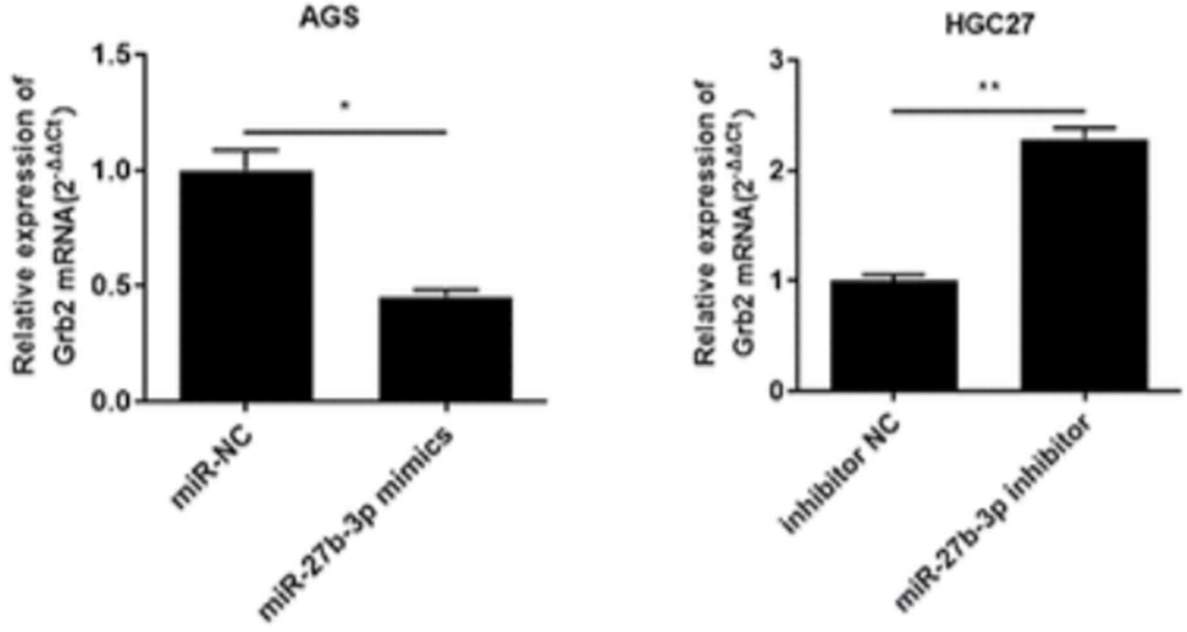

Figure 4: GRB2 expression when challenged with miR-27b-3p mimics (left) and inhibitors (right)

Next they quantified how much of the actual GRB2 protein was being expressed in the patients' cancer tissue versus their normal tissue via western blot. The results show that GRB2 protein expression is elevated in cancerous tissues versus normal gastric tissues from the same patient.

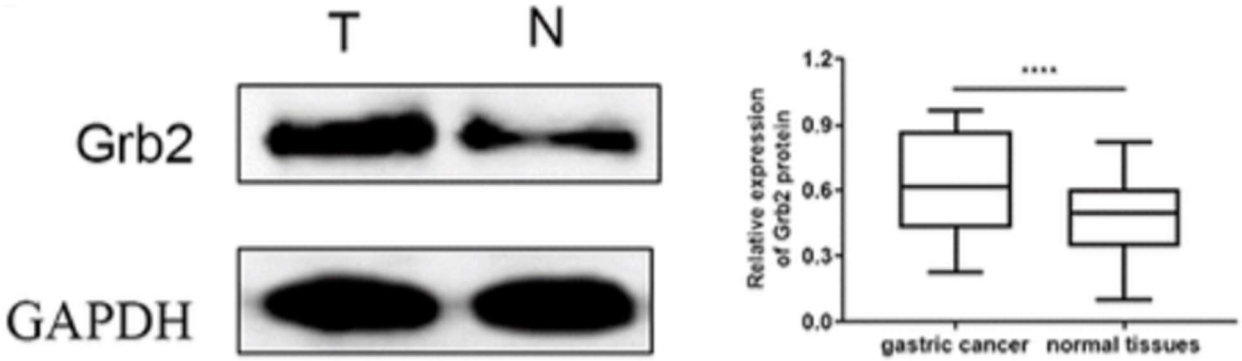

Figure 5: Western blot analysis of cancerous (T) and normal (N) patient tissue.

They conclude this section by performing a Pearson's correlation analysis on the cancerous patient samples using relative expression of GRB2 mRNA and miR-27b-3p as variables. Their results show that GRB2 mRNA expression is negatively correlated with miR-27b-3p expression.

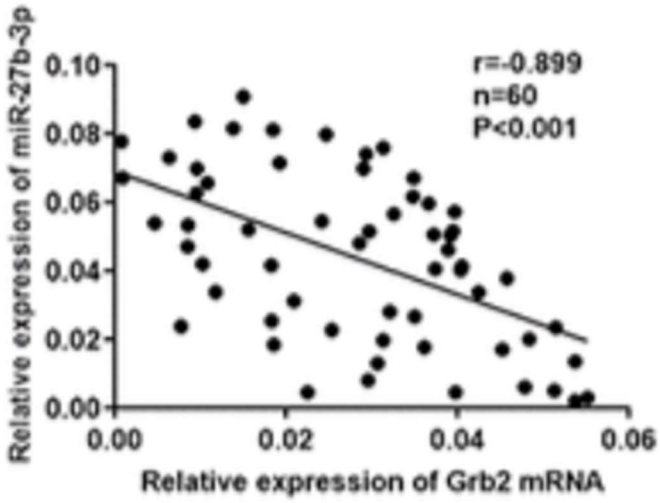

Figure 6: Pearson's correlation using 60 cancerous patient tissue samples

They go on to test how varying GRB2 levels affects metastatic behavior of the cancer cells and further prove the relationship between PAX4, miR-27b-3p, and GRB2. Their conclusion is that they discovered a new signaling pathway responsible for gastric cancer and it has the potential to reveal new biomarkers for gastric cancer progression.

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