Biomarker-Directed Therapy for Invasive Gastric Cancer: Characterization of a novel regulator of PI3K/AKT/mTOR signaling

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Abstract

Background: Gastric cancer (GC) is the second leading cause of cancer related death worldwide with poor clinical prognosis and limited current treatment options with a "one-size fits all approach". Dysregulation of the Akt/mTOR pathway is a common event in GC with *PIK3CA* mutations been reported to correlate with poor prognosis. Although, development of effective dual inhibitors is still premature, dual Akt/mTOR inhibitors are gaining immense interest owing to their advantage of effectively turning off this pathway and overcoming any feedback inhibition normally observed with single inhibitors.

Methods: Tissue microarray of two GC cohorts were used in correlation analysis between DP103 and PIK3CA expression. A series of *in vitro* experiments were carried out to validate the link between DP103 and Akt/mTOR signaling.

Results: Our lab previously identified DP103 to be significantly upregulated in breast tumor tissues compared to normal breast tissues. Herein, we provide clinical evidence showing increased expression of DP103 in GC patients' tissues compared to normal gastric tissues. In addition, we show a positive correlation between DP103 and PIK3CA expression in GC patients. Through *in vitro* DP103 knockdown studies conducted in a high DP103 GC cell line we showed substantial inhibition of Akt/mTOR pathway and its downstream targets important for cell survival both in a dose and time dependent manner upon dual Akt/mTOR inhibitor treatment.

Conclusion: This study proposes DP103 as a novel predictive biomarker for response to dual Akt/mTOR inhibitors in invasive GC, where Akt/mTOR pathway is often constitutively activated.

Results

1. DP103 is a prognostic marker in breast cancer

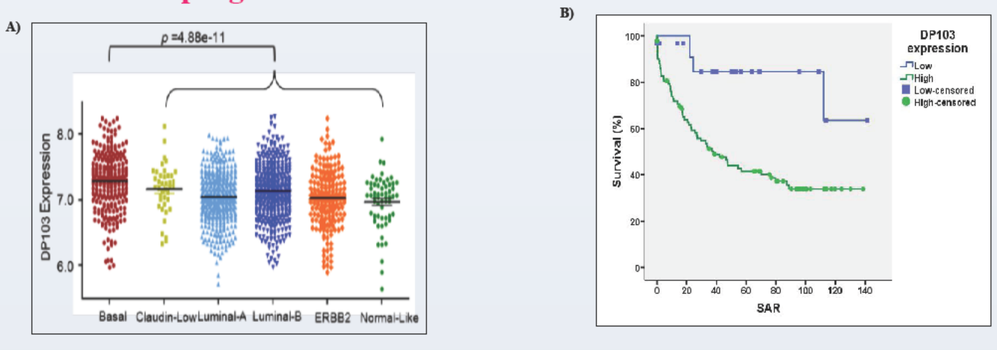

Figure 1.RNA helicase DP103 is highly expressed in invasive TNBCs

A) DP103 expression profile obtained from breast cancer tumors were collected and complied from public database Gene Omnibus (n=1325) across breast cancer subtypes and compared, where basal subtype was found to have significantly higher DP103 expression (Mann-Whitney test p=4.88e-11). B) Higher DP103 expression was associated with poorer survival, where OS = Overall Survival.

2. DP103 is upregulated in gastric tumors

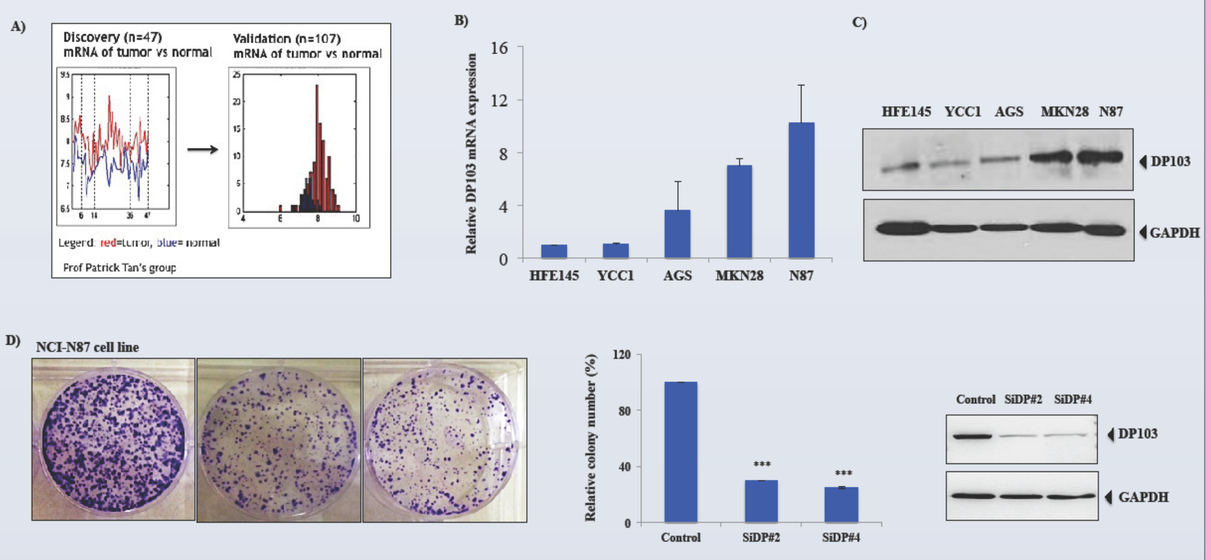

Figure 1.DP103 is significantly upregulated in gastric cancers playing an important role in survival in invasive forms of gastric cancer

A) DP103 gene is significantly upregulated in gastric tumors in comparison to normal gastric cells (mRNA) in both datasets validation (n=47) and discovery (n=107) (Prof Patrick Tan group). B & C) Basal DP103 expression was analyzed *in vitro* in a panel of gastric cancer (GC) cell lines where invasive gastric cancer cell lines , MKN-28 and N87 had higher DP103 expression both at mRNA and protein levels in comparison to GC cell lines obtained from primary tumor source (non-invasive YCC1 and AGS) as well as normal gastric cells (HFE145). D). The effect of knockdown of DP103 on cell proliferation was analyzed by relative colony number. Cell proliferation significantly (P<0.001) decreased in this long term colony forming assay in comparison to control group indicating that deletion of DP103 had a significant influence on the survival of NCI-N87 cells.

3. Positive correlation between DP103 and Akt/mTOR pathway associated genes

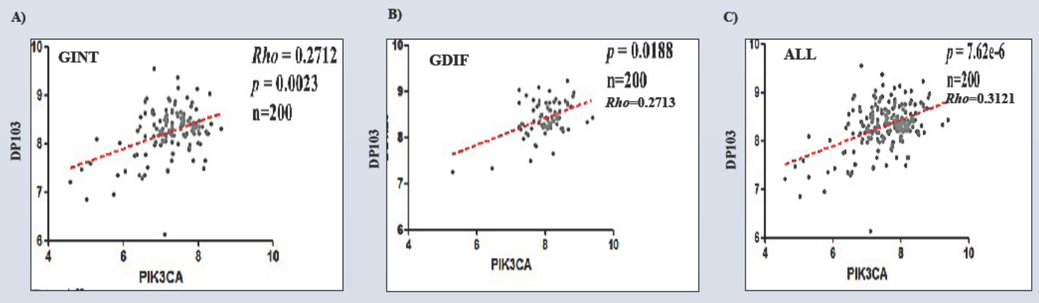

Fig3. Correlation between DP103 and PIK3CA gene expression levels

There is a statistically significant correlation between PIK3CA gene expression and the two molecular subtypes of gastric cancer (Fig A-C). Legends: GDIF: Diffuse type gastric cancer; GINT: Intestinal type gastric cancer

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Results

4. DP103 knockdown decreases cell proliferation by induction of apoptosis with NVP-BEZ235 treatment

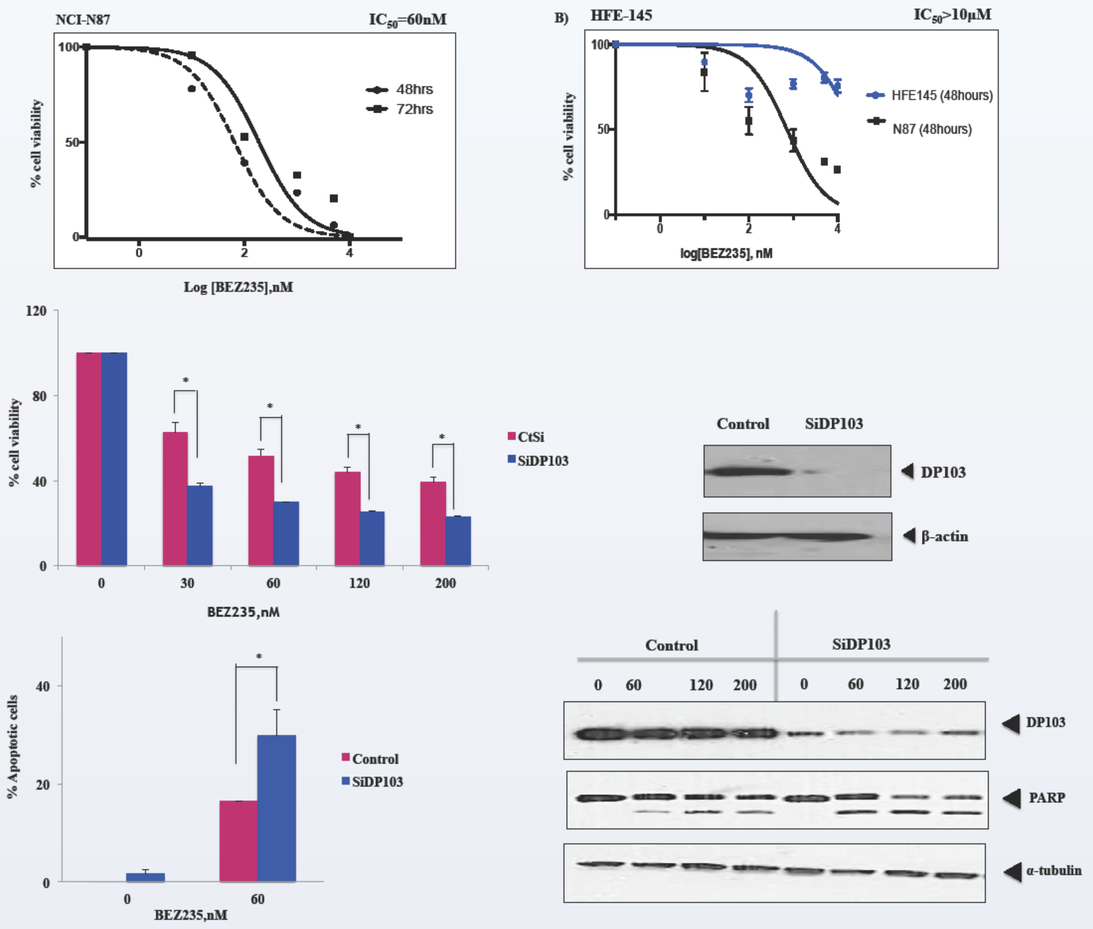

Figure 4.siDP103 depletion results in an increased apoptotic death with NVP-BEZ235 drug treatment

A) MTT assay shows NVP-BEZ235 inhibitory effect on NCI-N87 GC cell line with an IC_{50} of 60nM for 48hours . NVP-BEZ235 does not show an inhibitory effect on normal gastric cells (HFE-145). B) Downregulation of DP103 significantly suppressed cell proliferation upon drug treatment in a dose-dependent manner resulting in an increased total apoptotic death as compared to only drug treated cells as shown by Annexin/FITC apoptosis assay and western blot analysis for increased PARP cleavage.

5. siRNA mediated DP103 knockdown results in downregulation of the Akt/mTOR pathway

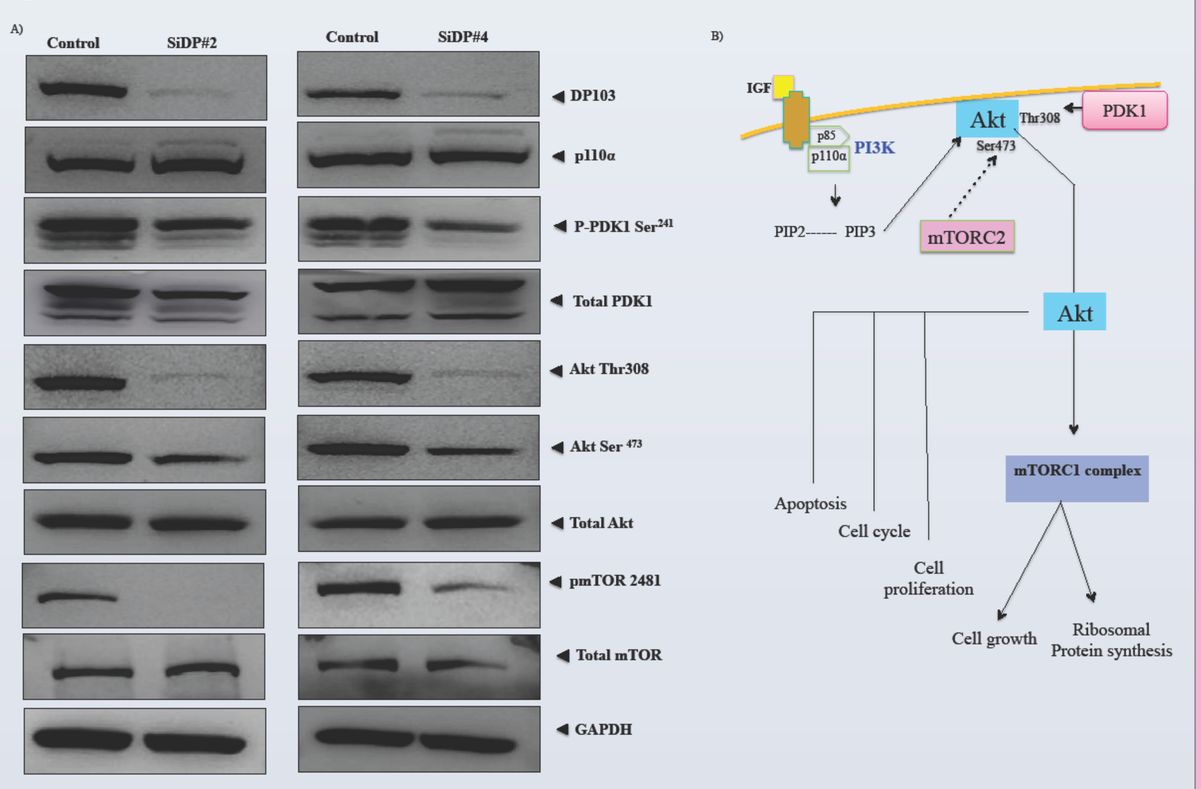

Figure 5. DP!03 knockdown results in decrease in the phosphorylation of the PI3K/Akt/mTOR pathway proteins

A) DP103 knockdown using tow different oligomers showed a significant decrease in the phosphorylation status of both Akt and mTOR pathway proteins indicating DP103 playing an important role in regulating this pathway.

6. DP103 interacts with p110α catalytic subunit of PI3K of the PI3K/Akt/mTOR pathway

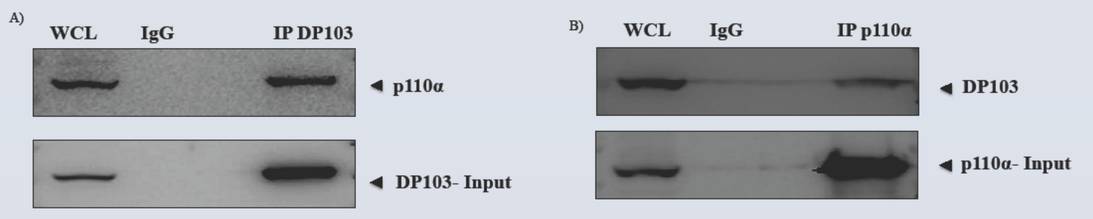

Figure 5. DEAD Box protein DP103 interacts with the p110α catalytic subunit of PI3K kinase of the Akt/mTOR pathway

A) IP DP103 pull down assay showed p110α to interact with DP103 , conversely p110α pull down assay also showed DP103 interacting with p110α , the catalytic subunit of PI3K kinase upstream of the Akt/mTOR pathway (B).

Conclusion

The above work summarizes the potential role of DP103 in PI3K/Akt/mTOR signaling, a pathway that has been significantly upregulated in GC. DP103 mRNA levels were significantly upregulated in GC tumor tissues. Further, DP103 depleted GC cells when treated with dual Akt/mTOR inhibitors showed an increased sensitization of the cells to apoptosis relative to only drug treated cells. Additionally, to asses if DP103 plays any role in regulating Akt/mTOR pathway , DP103 KD studies revealed decrease in the phosphorylation status of both Akt and mTOR pathway proteins. Interestingly, DP103 was found to interact with p110α, a catalytic subunit of PI3K , a kinase upstream of Akt , suggesting a novel role of DP103 in regulating PI3K/Akt/mTOR pathway in gastric cancer.

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