In silico identification of ADRA2A-associated miRNA expression as biomarkers for disease-free survival in breast cancer subtypes.

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Breast cancer is the most frequently diagnosed and leading cause of cancer death among women worldwide. An estimation of 2.300.000 women were diagnosed worldwide last year and around 685.000 women died from this disease during the same period.

Our group has been studying the adrenergic receptors in breast cancer for decades. In particular, when analyzing a compiled cohort extracted from GEO (1924 patients), we described that the expression of the α_{2A}-adrenergic receptor (ADRA2A) is an independent good prognostic factor in luminal tumors. Upregulated genes in ADRA2A high expression tumors were involved in cell–cell and focal adhesion, antiangiogenic processes and inhibition of cell proliferation. Downregulated genes in these tumors showed a strong enrichment in processes involved in cell division (DNA replication, G1/S transition and cell cycle, Br J Pharmacol 85: 2143, 2019).

The **objective** of the present research was to investigate if some ADRA2A-associated miRNAs could eventually be used as biomarkers for disease-free survival (DFS) in specific breast cancer subtypes.

Methods: miRNAs predicted to bind to the 3´UTR of the α_{2A}-adrenergic receptor (ADRA2A) mRNA were obtained with TargetScan Human, versión 7.2. The TCGA Breast Cancer (BRCA) database was used for interrogating ADRA2A and miRNA expression using Xena Functional Genomics Explorer. Only the patients with ADRA2A, miRNAs expression and intrinsic subtype information were selected. The cutoff points for disease-free survival (DFS) were selected using the Evaluate Cutpoint. Survival analysis was performed by Kaplan–Meier and log-rank (Mantel–Cox). GraphPad Prism 8.0.2 was used for the analysis and graphs.

TCGA: Correlation between ADRA2A and miRNAs able to bind to its 3´-UTR

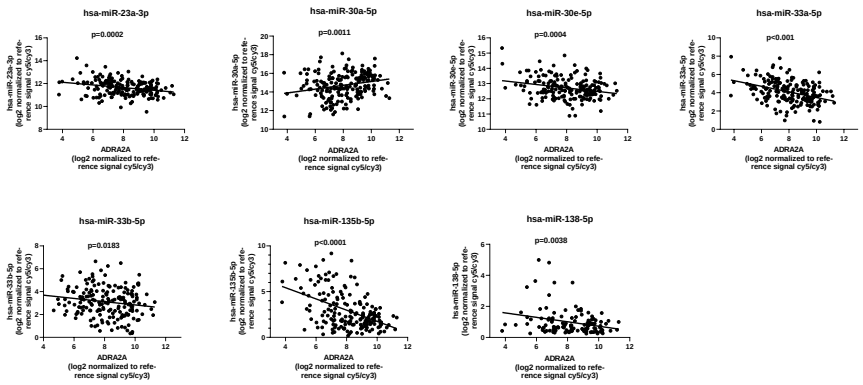

Figure 1: Correlation between ADRA2A and miRNAs that are predicted to bind to its 3´UTR using TargetScanHuman, versión 7.2. Only significant correlations are shown.

ADRA2A-associated miRNA expression by tumor subtype

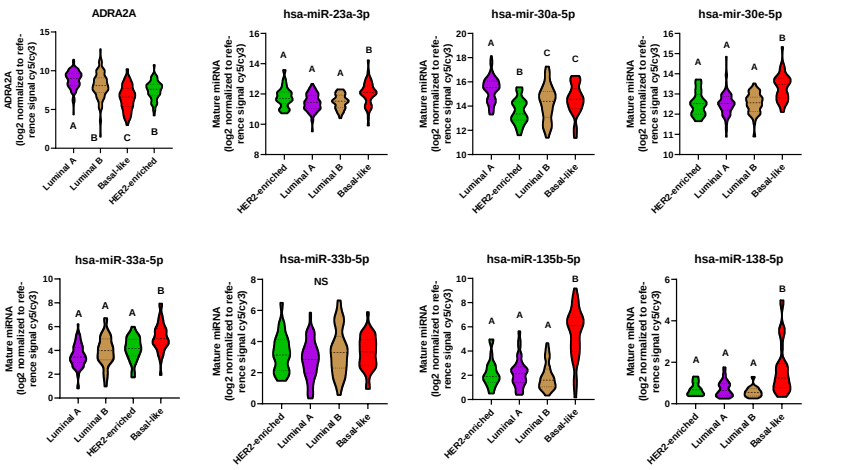

Figure 2: miRNA expression in the different breast cancer intrinsic subtypes of those miRNA which were found to correlate with ADRA2A expression. Several are overexpressed in basal-like tumors.

TCGA: Correlation between ADRA2A and miRNAs predictive of Disease-free survival

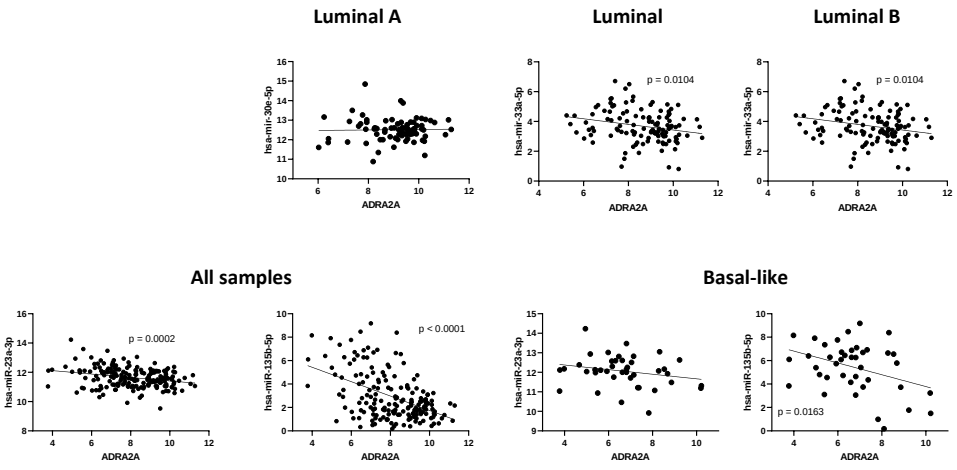

Figure 3: Correlation between ADRA2A and miRNAs that are predictive of DFS within the subtypes in which they are able to predict it.

TCGA: Predictive of Disease-free survival

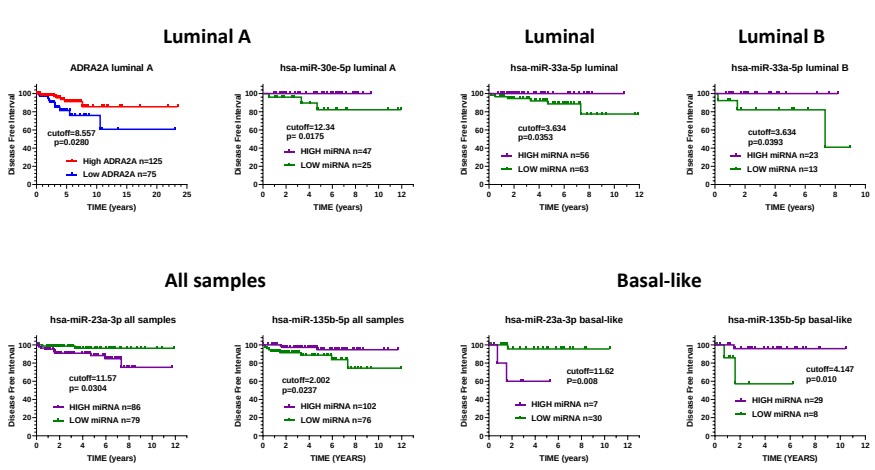

Figure 4: Kaplan-Meier analysis of DFS in different tumor subtypes. Only those predictive of DFS are shown.

Discussion: We verified in those patients from the TCGA cohort who included ADRA2A and miRNA expression as well as tumor information, that this receptor was predictive of better DFS survival. Even if the miRNAs were selected as those which could bind to ADRA2A mRNA 3´UTR, and within them those that correlated with its expression were further used, the only one that could be acting through a negative regulation of ADRA2A expression is hsa-miR23a-3p. This miRNA was found to negatively correlate with ADRA2A expression, and its low expression was predictive of better disease-free survival both in the whole cohort and in basal-like tumors. The other miRNAs, predictive of good DFS, do not seem to act through ADRA2A expression regulation since they do not correlate or are associated to a negative regulation while ADRA2A is predictive of better DFS. They are however very interesting because a high expression of hsa-miR 30e-5p is highly predictive of better DFS in luminal A tumors, hsa-miR-33a-5p for luminal in general and luminal B in particular and finally, hsa-miR-135b-5p for both the whole cohort and basal-like tumors.

Conclusion: Several miRNAs were found to significantly discriminate between patients with better and worse DFS in specific breast cancer subtypes and can therefore be selectively used as prognostic biomarkers in these tumor subtypes. However, the mechanisms involved still remain unclear and require further research.