Domanda estratta n. 1

AVVISO PUBBLICO, PER TITOLI E COLLOQUIO, PER L'ASSUNZIONE A TEMPO DETERMINATO DI N. 1 RISORSA NEL PROFILO DI RICERCATORE SANITARIO, CATEGORIA DS, DA ASSEGNARE ALLA UOC RICERCA TRASLAZIONALE ONCOLOGICA NELL'AMBITO DEL PROG. DAL TITOLO: "MIRNA-BASED THERAPEUTIC APPROACH TO FIGHT TRIPLE NEGATIVE BREAST CANCER" COD. PNRR-POC-2023-12377476, CUP H53C24000250001 - RESP. DR.SSA ANNAMARIA BIROCCIO

3 febbraio 2025, h. 12:00

Colloquio - Prova Tecnica

1) I microRNA (miRNA): definizione, processi di maturazione, ruolo e meccanismo d'azione in riferimento alla rilevanza clinica di questa classe di molecole.

2) Caratteristiche del tumore triplo negativo e geni BRCA in riferimento all'impatto di questi geni in ambito clinico (valore predittivo, prognostico e terapeutico).

Domanda estratta n. 1

AVVISO PUBBLICO, PER TITOLI E COLLOQUIO, PER L'ASSUNZIONE A TEMPO DETERMINATO DI N. 1 RISORSA NEL PROFILO DI RICERCATORE SANITARIO, CATEGORIA DS, DA ASSEGNARE ALLA UOC RICERCA TRASLAZIONALE ONCOLOGICA NELL'AMBITO DEL PROG. DAL TITOLO: "MIRNA-BASED THERAPEUTIC APPROACH TO FIGHT TRIPLE NEGATIVE BREAST CANCER" COD. PNRR-POC-2023-12377476, CUP H53C24000250001 - RESP. DR.SSA ANNAMARIA BIROCCIO

3 febbraio 2025, h. 12:00

Colloquio - Prova Informatica

1) Cos'è un Database

2) Principali funzioni dell'applicazione Excel

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RESEARCH

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MicroRNAome profiling of breast cancer unveils hsa-miR-5683 as a tumor suppressor microRNA predicting favorable clinical outcome

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Abstract

Background Breast cancer is a heterogeneous disease with diverse molecular subtypes, underscoring a better understanding of its molecular features and underlying regulatory mechanisms. Therefore, identifying novel prognostic biomarkers and therapeutic targets is crucial for advancing the current standard of care for breast cancer patients.

Methods Ninety-six formalin-fixed paraffin-embedded (FFPE) breast cancer samples underwent miRNAome profiling using QIAseq microRNA library kit and sequencing on Illumina platform. Mature miRNA quantification was conducted using CLC Genomics Workbench v21.0.5, while Relapse-free survival (RFS) analysis was conducted using RStudio 2023.09.1. Gain-of-function studies were conducted using miRNA mimics, while the effects of miRNA exogenous expression on cancer hallmark were assessed using 2-dimentional (2D) proliferation assay, three-dimensional (3D) organotypic culture, and live-dead staining. TargetScan database and Ingenuity Pathway Analysis (IPA) were used for miRNA target identification.

Results Hierarchical clustering based on miRNA expression revealed distinct patterns in relation to PAM50 classification and identified miRNAs panels associated with luminal, HER2, and basal subtypes. hsa-miR-5683 emerged as a potential prognostic biomarker, showing a favorable correlation with RFS and suppressing tumorigenicity under 2D and 3D conditions in triple-negative breast cancer (TNBC) models. Findings were further extended to the MCF7 hormone receptor positive (HR+) model. Transcriptomic profiling of hsa-miR-5683 overexpressing TNBC cells revealed its potential role in key oncogenic pathways. Integration of downregulated genes and CRISPR-Cas9 perturbational effects identified ACLY, RACGAP1, AK4, MRPL51, CYB5B, MKRN1, TMEM230, NUP54, ANAPC13, PGAM1, and SOD1 as *bona fide* gene targets for hsa-miR-5683.

Conclusions Our data provides comprehensive miRNA expression atlas in breast cancer subtypes and underscores the prognostic and therapeutic significance of numerous miRNAs, including hsa-miR-5683 in TNBC. The identified

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gene targets unravel the intricate regulatory network in TNBC progression, suggesting promising avenues for further research and targeted therapeutic interventions.

Keywords Breast cancer, microRNA, Survival, Prognostic biomarker, Therapeutic target

Background

Breast cancer stands as the leading cause of cancer-related mortalities among women, with an estimated 297,790 new cases and 43,170 deaths in 2023 in the United States alone [1]. Similar statistics were also seen around the globe with more than 2.26 million new cases of breast cancer were reported in women in 2020 [2]. Despite recent advancements in the field, invasive breast carcinoma remains a significant health concern, where infiltrating ductal carcinomas (IDC) constitute 80% of invasive breast cancers, followed by invasive lobular carcinomas. In situ carcinomas are predominantly ductal, comprising 80%, with lobular types accounting for approximately 10%[3]. The age-standardized incidence rates (ASRs) of breast cancer continue to rise globally, with the Middle East and North Africa (MENA) region reporting ASRs ranging from 9.5 to 50 cases per 100,000 women annually. In Qatar, breast cancer is the most commonly diagnosed cancer among women. This emphasizes the urgency for a better understanding of the disease, and the identification of novel biomarkers and therapeutic targets [2, 4, 5].

Early detection plays a pivotal role in reducing mortality rates associated with breast cancer [6, 7]. However, despite numerous efforts to enhance early detection, a significant proportion of breast cancer patients are still diagnosed at an advanced stage [8]. While Oncotype DX [9] and MammaPrint [10] have demonstrated acceptable performances in predicting the prognosis and treatment benefit of early-stage breast cancer, the identification of additional predictors for relapse-free survival (RFS) could offer an opportunity for patient stratification and personalized therapy.

The impact of breast cancer subtypes on prognosis is substantial, with four main classifications: luminal A (LumA), luminal B (LumB), HER2, and basal, based on the differential expression of certain genes. Prognosis is notably better for patients with ER+and/or PR+and HER2- tumors, compared to those with HER2 overexpressing or basal [11, 12]. Efforts to classify breast tumors have led to the development of surrogate markers based on immunohistochemistry, with ER, PR, and HER2 being pivotal for stratification. The PAM50 classification has proven superior to immunohistochemistry-based surrogates, offering better prognostic and treatment prediction capabilities [13–15].

Non-coding RNA, particularly microRNAs (miRNAs), emerged over the past years as a key player in cancer progression and development, functioning primarily through posttranscriptional gene regulation. Their involvement in various human diseases, including cancer, highlights their potential role as tumor suppressors or oncogenes [16–18]. Our recent work has suggested a plausible role for miRNAs in shaping breast cancer subtypes [19]. In the current study, we characterized the miRNA catalogue in a cohort of breast cancer patients from the MENA region. We subsequently delineated the miRNA expression profile in relation to the PAM50 intrinsic subtypes and relapse free survival (RFS). Notably, our analysis revealed hsa-miR-5683 as a predictor of better prognosis. Mechanistically, the exogenous expression of hsa-miR-5683 suppressed breast cancer proliferation, cell cycle progression, and growth under three-dimensional (3D) conditions. Through integration with CRISPR-Cas9 essentiality data, our findings unveiled several crucial genes as *bona fide* targets for hsa-miR-5683, suggesting the potential utilization of hsa-miR-5683 as prognostic biomarkers and therapeutic targets, particularly for TNBC. Concordant with our data, miR-5683 was recently shown to promote apoptosis in gastric cancer [20].

Methods

Ethics statement and study cohort

The study received MRC-01–19–142 ethical approval from Hamad Medical Corporation (HMC) and QBRI-IRB 2020–09–035 approval from Qatar Biomedical Research Institute (QBRI). Clinicopathological features of the study cohort are presented in Table 1. Detailed description of the study cohort and inclusion/exclusion criteria can be found in our recent publication [19].

Total RNA isolation and next-generation sequencing (NGS)

Total RNA isolation from FFPE tissues was conducted as we described before [19]. For miRNA library preparation, 100 ng of total RNA was used as input for 3′ ligation, followed by 5′ ligation and reverse transcription using the QIAseq miRNA library kit (QIAGEN, Hilden, Germany). The resulting cDNA libraries were quantified using the Qubit dsDNA HS assay kit and assessed for size distribution using the Agilent 2100 Bioanalyzer DNA1000 chip. Pooled libraries underwent sequencing on the Illumina platform. The miRNA transcriptomic data were deposited in the SRA repository under BioProject number PRJNA953015. For miRNA analysis, FASTQ files were mapped to the miRBase v22 database and the miRNA expression (total counts) were calculated using the small RNA analysis workflow in CLC Genomics Workbench 21.0.5. Expression data were then imported into