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ARTICLE

In Silico Study Predicts CCDC69 as a Novel Tumour Suppressor Gene in HER2+ Breast Cancer

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ABSTRACT

Differential gene expression analysis using databases followed by overall survival (OS) analysis is currently used to identify different oncogenes and tumour suppressor genes. The present study identified coiled coiled domain containing protein 69 (CCDC69) as a tumour suppressor gene in breast cancer by differential gene expression analysis using TCGA dataset for breast adenocarcinoma (BRCA) followed by OS and relapse free survival (RFS) analysis using Kaplan Meier (KM) plotter tool. CCDC69 was observed to be down regulated in tumour of breast cancer patients in BRCA. Following OS analysis for different breast cancer sub-types, low expression of CCDC69 has been observed to be associated with poor survival in HER2+ breast cancer only. CCDC69 was also found to be down regulated in different HER2+ breast cancer cells by analysing Gene Expression Omnibus (GEO) database. Additionally, CCDC69 was found to be under expressed in single cell HER2 positive population, which is evident from the single cell expression ATLAS database. Furthermore, CCDC69 has been observed to be lowly expressed with overexpression of HER2 in breast cancer by co-expression study. The possible mechanism of CCDC69 down regulation in HER2+ breast cancer was resolved using P-SCAN tool. P-SCAN analysis suggested a group of transcription factors (TFs) among which androgen receptor (AR) has been selected as the probable TF that could play a role in CCDC69 down regulation in HER2+ breast cancer. Moreover, overexpression of AR has been observed in BRCA and HER2+ single cell population. AR has also been observed to be co-expressed positively with HER2, but negatively with CCDC69 in breast cancer. Down regulation of CCDC69 can be predicted to stabilize microtubule formation following stimulation of cell growth and cell migration leading to HER2+ breast cancer progression and metastasis.

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Abbreviation

CCDC69: Coiled Coiled Domain Containing protein 69; TCGA: The Cancer Genome Atlas; BRCA: Breast Adenocarcinoma; GEPIA: Gene Expression Profiling Interactive Analysis; MTCI: Molecular Therapeutics for Cancer, Ireland; GEO: Gene Expression Omnibus; OS: Oveall Survival; RFS: Relapse Free Survival

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1. Introduction

Breast cancer is a rapidly growing and the most predominant disease of cancer mediated morbidity and mortality in women all over the world. The possibility of recurrence of breast cancer depends on the tumour size, grade, nodal status, tumour subtype and especially mode of therapy [1]. The presence of hormonal receptors "estrogen receptor (ER) and progesterone receptor (PR)" and HER-2 "human epidermal growth factor receptor 2" receptor in the tumour cells have been selected as one of the important factors for the diagnosis of breast cancer. Based on the expression of these receptors, breast cancer has been divided into different sub-types: luminal A, luminal B, triple negative and HER2 positive [2]. Over the past decade, advancement with cancer research using different databases, high throughput technologies, clinical trial allowed the discovery of new drugs for treatment of breast cancer. Better understanding of phenotype and heterogeneity of breast cancer has opened the possibility for the development of more effective and individualized approach to treatment. The most common treatments that currently available are surgery, radiation therapy and chemotherapy. Surgery and radiation therapy are the most common treatment options for primary tumours and large metastases, while chemotherapy is the best treatment option for metastatic tumours. Traditional anticancer chemotherapeutic drugs inhibit cancer cell division and DNA replication leading to prevention of breast cancer. However, they do not seem to be appropriate in all types of cancer. Regarding the background and disadvantage of chemotherapy, complementary treatment modalities are being widely explored in recent years. For example, molecular therapy, anti-angiogenesis therapy, immunotherapy, apoptosis regulation, signal-transduction therapy, targeted radionuclide therapy and nucleic acid based therapies have attracted attention from the health care system [3]. Therefore, finding new therapeutic targets such as oncogenes, tumour suppressor genes, signalling molecules are the recent trends of research for targeted therapy to combat cancer. Targeted therapy exerts its anticancer effects through multiple mechanisms, including arrest of cell growth, induction of apoptosis, and suppression of spreading of tumour (a.k.a tumour metastasis) as well as immune function regulation and multidrug resistance reversal.

Differential gene expression analysis using different databases followed by OS is important in the cancer research. This can help in identifying different oncogenes and tumour suppressor genes. The aim of our study was to identify a novel tumour suppressor gene in breast cancer. Herein, we have identified CCDC69 as a tumour suppressor gene in breast cancer by differential gene expression analysis using TCGA dataset for BRCA followed by OS and RFS analysis using KM plot and CCDC69 has been found to be down regulated in HER2+ breast cancer. Transcription factor, AR was predicted by our *in silico* study as the key regulator for the down regulation of CCDC69 in HER2+ breast cancer.

2. Methods and Tools

2.1 Differential Gene Expression Analysis Using Gene Expression Profiling Interactive Analysis (GEPIA) Tool

GEPIA allows users to input custom statistical methods and thresholds for TCGA dataset to dynamically obtain differentially expressed genes. The differential gene expression analysis for the present study has been done using GEPIA online server tool. Details of the method are described in the GEPIA website [4].

2.1 Overall Survival (OS) Analysis Using Kaplan Meier Plotter

The Kaplan Meier plotter is capable to assess the effect of 54k genes on survival in 21 cancer types. The largest datasets include breast (n=6,234), ovarian (n=2,190), lung (n=3,452), and gastric (n=1,440) cancer. Details of the method are described in the KM plotter website [5].

2.3 MTCI Breast Cancer Overall Survival Tool

Molecular Therapeutics for Cancer, Ireland (MTCI) is a Science Foundation Ireland-funded strategic research cluster which aims to discover and develop new anti-cancer drugs. Details of the method are described in the MTCI website.

2.4 Single Cell Expression Atlas - EMBL-EBI

EMBL-EBI Single Cell Expression Atlas, an open public repository of single cell gene expression data. Details of the method are described in the Single Cell Expression Atlas - EMBL-EBI website.

2.5 Statistical Analysis

Statistical analyses were followed according to the analysis has been done in different tools that are used in the present study. Details of the method are available in the website of the tools.

3. Results and Discussion

3.1 Differential Gene Expression Analysis Identifies CCDC69 as a Tumour Suppressor Gene

To find out a novel tumour suppressor gene in breast

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cancer, we have first undertaken differential gene expression analysis (log2 fold change cut off and p value were considered ≤-2 and ≤.001 respectively) for BRCA in TCGA dataset using GEPIA (Gene Expression Profiling Interactive Analysis) tool followed by OS and RFS analysis using KM-Plotter. We have selected CCDC69, which may act as a tumour suppressor in breast cancer following literature study since we did not find significant number of publications that suggests CCDC69 role as tumour suppressor in breast cancer. CCDC69 has been observed to be down regulated in different types of cancer including breast in TCGA dataset (Figure 1 A-C). Especially, CCDC69 mRNA was under expressed in breast tumour sample (~6 fold compared to normal) in BRCA in TCGA dataset (Figure 1D). OS analysis and RFS analysis for breast cancer using KM-Plot suggested low expression of CCDC69 is associated with poor survival with a p value of $8.2e^{-07}$ and $7.8e^{-16}$, respectively, which predicts that CCDC69 may strongly acts as a tumour suppressor gene in breast cancer (Figure 2A).

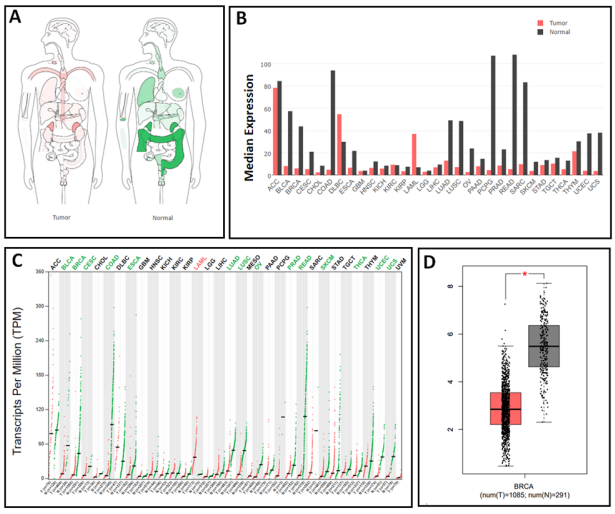

Figure 1

Figure 1. CCDC69 expression in different cancers

3.2 OS Analysis for Different Breast Cancer Subtypes Predicts that CCDC69 may Play Role only in HER2+ Breast Cancer

Although low expression of CCDC69 expression was found to be associated with poor survival in breast cancer, it is important to know in which breast cancer sub-type CCDC69 may play role as a tumour suppressor. To this end, OS analysis has been performed for different breast cancer subtypes using MTCI breast cancer survival analysis tool. CCDC69 expression was not found to be associated with poor survival in LUMA, LUMB and triple negative breast cancer subtypes (p values are 0.216, 0.656 and 0.677 respectively); however, low expression of CCDC69 was found to be associated with poor survival in HER2+ breast cancer population with a p value of 0.05 (Figure 2B). Next to determine whether CCDC69 is under expressed in HER2+ breast cancer cell lines, we used GEO dataset to see CCDC69 mRNA expression in different breast cancer cells. Our study revealed that CCDC69 is down regulated in different HER2+ positive breast cancer cell lines (Figure 3A). We have also checked CCDC69 expression in single cell population isolated from breast cancer patients using single cell expression ATLAS database. Interestingly, CCDC69 was found very lowly expressed in HER2+ single cell population (Figure 3B). Additionally, we determined whether CCDC69 is co-expressed with HER2 in breast cancer by co-expression analysis and the

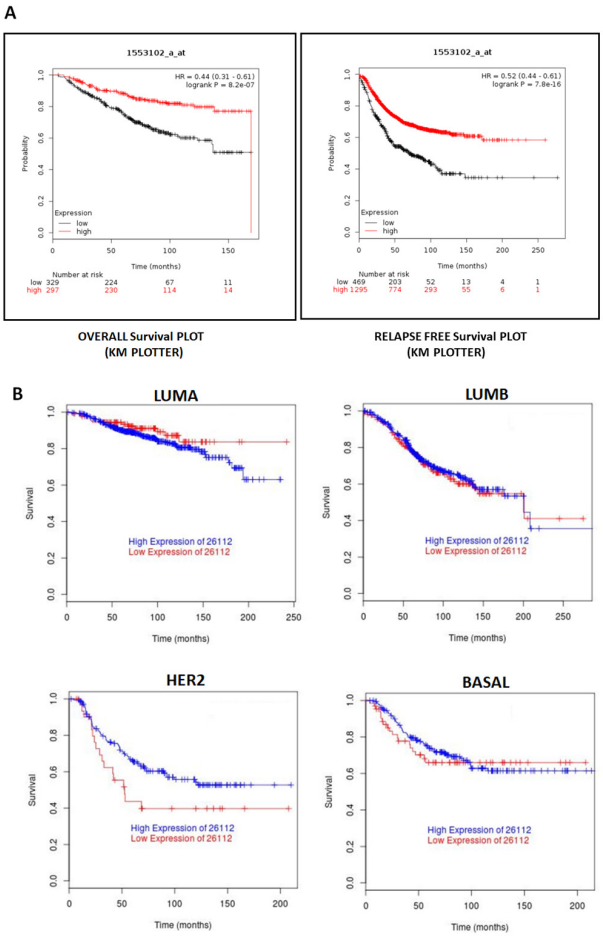

Figure 2

Figure 2. A) Overall survival analysis plot of CCDC69 in breast cancer; B) Overall survival analysis plot of CCDC69 in different breast cancer sub-types

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study indicated that CCDC69 is co-expressed negatively with HER2 (Figure 3C). Overall, the above observations strongly support CCDC69 down regulation in HER2+ breast cancer cells.

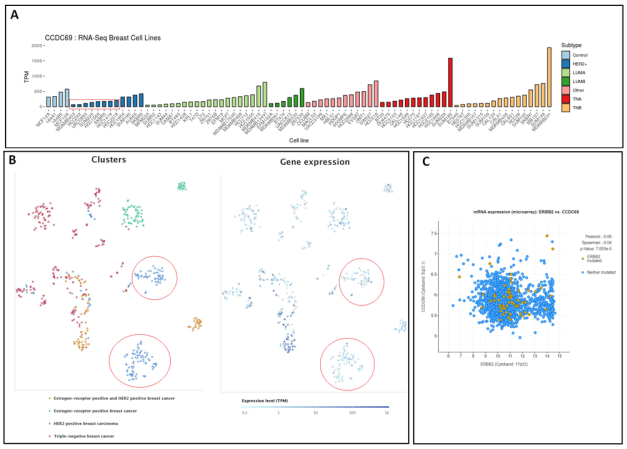

Figure 3

Figure 3. CCDC69 expression in different breast cancer cell lines and single cell population

3.3 Role of CCDC69 Co-expressed Genes in Breast Cancer

Gene co-expression analysis is used to identify the function of an unknown gene in biological processes to prioritize the role of the candidate gene in diseases and to determine transcriptional regulatory programmes [6]. To gain an insight in the function of CCDC69 in biological process or in disease regulation, we did co-expression study with the use of genevestigator tool. Co-expression analysis revealed 25 genes which are further analysed for their role in breast cancer using TCGA dataset for BRCA (Figure 4A). Unfortunately, expression of not a single gene among the 25 genes was found to be changed significantly in tumour tissue compared to normal tissue in TCGA dataset (Figure 4B). Therefore, it may be concluded that CCDC69 is down regulated exclusively in BRCA in comparison to its co-expressed genes. Next to determine the function of CCDC69 or its role in signalling pathways, we have used gene analytics tool. No signalling pathways, however, has been observed where CCDC69 appears to be involved and could play a significant role leading to breast cancer progression. Nevertheless, literature study indicated a role of CCDC69 in the activation of p14ARF/MDM2/p53 signalling pathway in ovarian cancer [7]. Thus, in vitro study that shows a functional role of CCDC69 in breast cancer will be required in future.

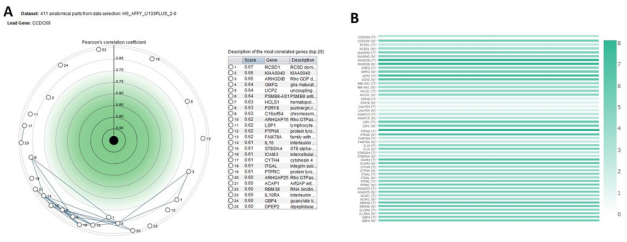

Figure 4

Figure 4. CCDC69 co expressed genes and their role in breast cancer

3.4 AR may be Involved in the down Regulation of CCDC69 in HER2+ Breast Cancer

Since CCDC69 was found to be down regulated in HER2+ breast cancer and as OS analysis strongly supports its role as a tumour suppressor; therefore it appears essential to know the mechanism by which CCDC69 is down regulated. To this end, P-SCAN analysis was performed to identify possible transcription repressors that may bind to the CCDC69 promoter region leading to suppression of CCDC69 mRNA transcription. P-SCAN analysis identified top 15 transcription factors with significant p value, of which androgen receptor (AR) was selected as the probable regulator of CCDC69 (Figure 5A). AR was selected to cause CCDC69 mRNA down regulation in HER2+ breast cancer because AR was found to be co-expressed with HER2 positively; however, negatively with CCDC69 by co-expression analysis (Figure 5B & C). Additionally, AR was found to be overexpressed in the tumour of breast cancer patients TCGA dataset for BRCA (Figure 5D). Interestingly, gene expression analysis for AR in single cell dataset suggests that AR is overexpressed in HER2+ breast cancer subtype (Figure 5E).

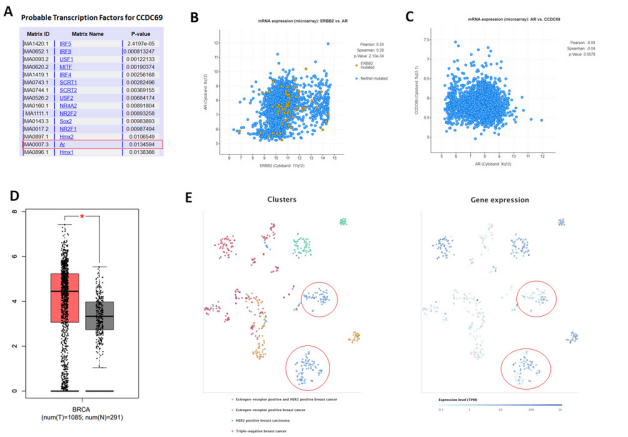

Figure 5

Figure 5. Mechanism of CCDC69 down regulation in HER2+ breast cancer

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Transcription factors can act both as a transcriptional activator or repressor depending on the cell system. AR has been indicated to act as a transcriptional repressor in a recently published article [8]. Therefore, it is suggested that overexpressed AR may bind to the CCDC69 promoter leading to the transcriptional repression of CCDC69 in HER2+ breast cancer.

4. Conclusion and Future Prospects

The present in silico study identifies CCDC69 as a novel tumour suppressor gene in HER2+ breast cancer. The probable mechanism for CCDC69 down regulation in HER2+ breast cancer was resolved and AR is suggested as the predominant transcriptional repressor for the under expression of CCDC69 mRNA in HER2+ breast cancer. The present study will open the possibility of a wet lab work to verify the role of CCDC69 as a tumour suppressor in HER2+ breast cancer. The present study also suggests that AR can be a therapeutic target in HER2+ breast cancer and its inhibition can be considered as a therapeutic strategy in HER2+ breast cancer treatment. Functional study of CCDC69 and identifying the mechanism by which CCDC69 play a role in HER2+ breast cancer are also seem to be promising for future study. Recent study demonstrated that CCDC69 can destabilize microtubules [9]. Knockdown of CCDC69 by RNAi leads to the formation of aberrant central spindles and interferes with the localization of midzone components such as aurora B, PRC1, MgcRacGAP, and Plk1 leading to stabilization of microtubules [9]. It is well known that microtubules stabilization induces cell proliferation and migration [10,11]. Conceivably, it can be speculated that down regulation of CCDC69 may stabilize microtubule formation and subsequently stimulates cell growth and migration leading to breast cancer progression and metastasis. Hence, overexpression of CCDC69 in HER2+ breast cancer may be a therapeutic strategy for the treatment in future.

Conflict of Interest

There is no conflict of interest.

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