***In silico* analyses reveal new putative Breast Cancer RNA-binding proteins.**

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Abstract

Breast cancer (BC) is the leading cause of cancer-associated death among women worldwide. Despite treatment efforts, advanced BC with distant organ metastases is considered incurable. A better understanding of BC molecular processes is therefore of great interest to identify new therapeutic targets. Although large-scale efforts, such as The Cancer Genome Atlas (TCGA), have completely redefined cancer drug development, diagnosis, and treatment, additional key aspects of tumor biology remain to be discovered. In that respect, post-transcriptional regulation of tumorigenesis represents an understudied aspect of cancer research. As key regulators of this process, RNA-binding proteins (RBPs) are emerging as critical modulators of tumorigenesis but only few have defined roles in BC. To unravel new putative BC RBPs, we have performed *in silico* analyses of all human RBPs in three major cancer databases (TCGA-Breast Invasive Carcinoma, the Human Protein Atlas, and the Cancer Dependency Map project) along with complementary bioinformatics resources (STRING protein-protein interactions and the Network of Cancer Genes 6.0). Thus, we have identified six putative BC progressors (MRPL13, SCAMP3, CDC5L, DARS2, PUF60, and PLEC), and five BC suppressors RBPs (SUPT6H, MEX3C, UPF1, CNOT1, and TNKS1BP1). These proteins have never been studied in BC but show similar cancer-associated features than well-known BC proteins. Further research should focus on the mechanisms by which these proteins promote or suppress breast tumorigenesis, holding the promise of new therapeutic pathways along with novel drug development strategies.

Introduction

Breast cancer (BC) is the leading cause of cancer-associated death (15%: 626,679 cases) and the most commonly diagnosed cancer (24%: 2,088,849 cases) among women worldwide¹. BC is characterized by a complex interaction between environmental factors and biological aspects, such as gene deregulation, hormone disruption or ethnicity²⁻⁴. Despite subtype-specific treatment efforts, advanced BC with distant organ metastases is considered incurable². Therefore, a better understanding of BC molecular processes is still of great interest to identify new therapeutic targets.

Current oncological research generates large-scale datasets that harbor essential aspects of tumor biology. For instance, The Cancer Genome Atlas (TCGA), with over 2.5 petabytes of data, has molecularly characterized over 20,000 patient samples covering 33 cancer types⁵⁻¹⁰. Also, The Cancer Dependency Map (DepMap) project, using loss-of-function genetic screens, has identified essential genes for cancer proliferation and survival *ex vivo*¹¹⁻¹³. Additionally, The Human Protein Atlas (HPA) constitutes a comprehensive resource to explore the human proteome in healthy and tumor tissues¹⁴⁻¹⁶. Although these datasets have completely redefined cancer drug development, diagnosis, and treatment, additional key aspects of tumor biology remain to be discovered. In that respect, post-transcriptional regulation of tumorigenesis represents an understudied aspect of cancer research¹⁷.

As key regulators of post-transcription, RNA binding proteins (RBPs) are particularly relevant due to their implication in every post-transcriptional step of gene expression: RNA splicing, transport, stability, translation, and localization. As a result, genomic alterations of these proteins lead to dysfunctional cellular processes; indeed, RBPs are emerging as critical regulators of tumorigenesis but only few have well-defined roles in BC¹⁸⁻²⁶. To date, 1,393 RBPs have been experimentally identified from human RNA interactomes²⁷. Despite efforts to understand their role in cancer^{28,29}, an integrated analysis of the aforementioned databases along with other *in silico* approaches is still missing in BC. To shed light on this matter, we analyzed and integrated RBPs genomic alterations, protein–protein interaction (PPI) networks, immunohistochemical profiles, and loss-of-function experiments to unravel new putative BC RBPs.

Results.

An Overview of RBPs genomic alterations in BC.

To globally assess the potential role of RBPs in BC versus well-known BC genes, we first interrogated the Breast Invasive Carcinoma (TCGA, PanCancer Atlas)⁵⁻¹⁰ database for genomic alterations of RBPs (n=1392), BC genes (n=171)³⁰ and non-cancer genes (n=170)³¹ (Supp. Table 1). As shown in Fig. 1A, both genomic alterations frequencies of RBPs and BC genes were significantly higher than the one observed for non-cancer genes. Interestingly, RBPs present a similar amount of genomic alterations than BC genes (Fig. 1A), highlighting the putative role of RBPs in BC.

To obtain insights into how these proteins are altered in BC, we catalogued their genomic alteration types. As shown in Fig. 1B and Supp. Table 2, most genomic alterations are related to an overrepresentation of the mRNA (68.7%) or gene loci (15.4%).

Identification of highly altered BC RBPs

To identify BC-related RBPs, we next interrogated The Network of Cancer Genes 6.0 (NCG6)³⁰ for RBPs having known or predicted cancer driver roles. NCG6 harbors the most recent catalog of cancer driver genes³⁰. Thus, we identified 225 RBPs: 14 implicated in BC (2 oncogenes, 4 tumor suppressors, and 8 unknown), indicating that these proteins remain poorly studied in breast carcinogenesis, and 211 related to other cancer types (21 oncogenes, 24 tumor suppressors, and 166 unknown) (Fig. 2A, Supp. Table 3).

To unravel putative RBPs implicated in tumor progression or suppression, we analyzed RBPs genomic alterations based on their progressor or suppressor profiles. Tumor progressors tend to be overexpressed (mRNA upregulation or genomic amplification), while suppressors are downregulated (mRNA downregulation or genomic deletion)³². Gene mutations or fusions have been observed in both scenarios. On this basis, we identified highly altered BC RBPs (Table 1, Supp. Table 2). For instance, MRPL13 molecular and cellular functions have never been studied in BC; however, MRPL13 has shown to interact with the estrogen receptor 2 (ESR2), a tumor suppressor protein in breast and other cancers³³. Interestingly, 30% of all human RBPs interact with the tumor suppressor ESR2³³ (Fig. 2B). We also found known BC progressors and suppressors proteins, such as DAP3¹⁸, MTDH¹⁹ or CCAR2²⁰, validating our strategy (Table 1, Supp. Table 2). Also, these analyses reveal proteins that have not been related to tumorigenesis, and yet they are highly altered in BC (e.g. TFB2M, C1ORF131 or DDX19A) (Table 1, Supp. Table 2).

To further identify key RBPs implicated in BC, we analyzed RBPs genomic alterations by subtype (Normal-like, Luminal A, Luminal B, Her2-enriched, and Basal-like. Supp. Table 4) or staging (stage I to IV; Supp. Table 5). As shown in Fig. 2C, RBPs are highly altered in Basal-like BC compared to other subtypes. Concerning staging, RBPs seem to be more altered in stage IV. Some RBPs reached higher frequencies of genomic alterations per subtype (Fig. 2C, Supp. Table 4) or stage (Fig. 2D, Supp. Table 5). For instance, ARF1, the most altered protein in stage IV (Fig. 2D), has been shown to promote BC metastasis³⁴; PARP1 has also been demonstrated to enhance metastasis not only in BC³⁵ but also in other cancer types³⁶. In contrast, SCAMP3 and HEATR6 presented a similar amount of genomic alterations (Fig. 2D) and none of them has been previously studied in BC.

RBPs interact with well-known BC proteins

Networking analysis has proven to be useful in identifying RNA regulons and key tumoral proteins³⁷. On this basis, we next explored PPIs between RBPs (n=1392) and well-known BC proteins (n=171)³⁰ using STRING database³⁸. PPIs were obtained from experiments and databases (interaction score=0.9, highest confidence). We identified 113 BC proteins interacting with 398 RBPs (Supp. Table 6). By narrowing down our analysis to experimental interactions only (Fig. 3), we observed two main networks around SF3B1 and CDC5L proteins. According to g:Profiler³⁹, proteins interacting with SF3B1 are implicated in RNA splicing ($P_{adj}=3.783 \times 10^{-34}$; GO:0000377), while proteins connected to CDC5L are mainly involved in chromatin binding ($P_{adj}=1.500 \times 10^{-2}$; GO:0003682). We also observed proteins with both BC and RNA-binding features present in the two main networks: SF3B1, CTNNA1, RBMX, and SPEN. Additionally, 18 RBPs interact with at least one BC protein. Thus, we identified RBPs that may have a putative role in BC molecular pathways through PPIs.

Identification of differentially expressed RBPs in breast tumor tissues.

The Human Protein Atlas (HPA) constitutes¹⁴⁻¹⁶ a major effort to address protein expression in healthy and tumoral human tissues. We therefore identified RBPs having a differentially protein expression profile in breast tumor tissues. To this end, we compared immunohistochemical levels (not detected, low, medium and high) of 1212 available RBPs between normal and cancerous breast tissues (Fig. 4, Supp. Table 6). Most RBPs presented common immunohistochemical levels between both breast tissues: not detected (n=130), low (n=52), medium (n=366), and high (n=172) (Fig. 4A). Moderate protein expression

changes, defined by one variation level (e.g. not detected to low or medium to high) were observed in 406 RBPs.

To identify RBPs with highly altered protein expression profiles in tumor tissues, we categorized RBPs having a twofold variation level as upregulated or downregulated compared with normal tissues; thus, we identified 24 upregulated and 62 downregulated RBPs (Fig. 4A, Supp. Table 6). As expected, our approach revealed well-known BC proteins, such as KPNA2²¹ or G3BP2²², which validate our analysis. KPNA2 is highly expressed in BC tissues (7 out of 12 tumor samples are classified as high) (Fig. 4B, Supp. Table 6). On the contrary, G3BP2 expression is reduced in breast tumor tissues (Fig. 4B, Supp. Table 6). We also observed two RBPs that have never been studied in BC: DARS2 (overexpressed) and SUPT6H (downregulated) (Fig. 4B, Supp. Table 6).

Exploring RBPs BC dependencies

Most RBPs present numerous genomic alterations (Fig. 1; Fig 2C and D; Supp. Table 2-4), making it difficult to detect essential RBPs for tumor survival. To study RBPs BC dependencies, we analyzed 1288 available RBPs on CERES¹¹ and 1290 available RBPs on DEMETER2^{12,13} through the DepMap portal (<https://depmap.org/portal/>). Both initiatives report loss-of-function screens performed in several human cancer cell lines¹¹⁻¹³.

Fig. 5A shows the distribution of dependency scores of all available RBPs in 82 (DEMETER2^{12,13}) and 28 (CERES¹¹) BC cell lines. The genome-scale RNAi loss-of-function screens (DEMETER2^{12,13}) identified 90 essential RBPs (Fig. 5A), being SNRPD1, SF3B1, SF3B2, RPL5, ARCN1, EIF3B, RAN, COPB1, RPL14, and VCP (mean dependency scores ranging from -1.3 to -1.5) the top ten essential RBPs for BC survival (Supp. Table 8). On the other hand, genome-scale CRISPR-Cas9 loss-of-function screens (CERES¹¹) determined 176 essential RBPs (Fig. 5A), being RAN, HSPE1, SNRNP200, SNRPD1, SARS, EEF2, RPL37, CCT3, KPNB1, and RPL23 (mean dependency scores ranging from -1.5 to -1.8) the top ten essential RBPs for tumor survival (Supp. Table 9). *In toto*, 207 essential RBPs were identified by both computational methods (Fig. 5A; Supp. Table 8-9).

To identify essential RBPs per BC molecular subtype, we first updated subtypes by merging data from Smith *et al.*⁴⁰, Dai *et al.*⁴¹, and Kao *et al.*⁴² (Supp. Table 10). Next, we identified and compared 203 LumA, 96 LumB, 206 Her2, and 212 Basal essential RBPs (Fig. 5B; Supp. Table 8-9). Thus, we identified essential RBPs for each BC subtype: 7 LumA (HSPD1, UBE2M, SART3, USP36, GTPBP4, DHX33, and UPF1), 5 LumB (RPS21, GNL3L,

ZNF207, AQR, and RPL17-C18orf32), 7 Her2 (DDX39B, NMT1, ISY1, DARS, HEATR1, MAT2A, and SYF2), and 9 Basal (EIF3C, UTP20, TXN, NOP58, ALDOA, CCT2, NOP2, DDX54, and PRMT1) (Fig. 5B).

Unraveling putative BC RBPs

Cancer-related RBPs control hundreds of tumor mRNAs, interact with well-known cancer driver proteins and appear to be highly altered in cancer genomic databases and tumor tissues³². We therefore reasoned that integration of our previous analyses could narrow down the identification of potential BC RBPs.

To this end, we first focused on RBPs having putative tumor progression profiles. Thus, we overlapped our previous results as follows: 1) 348 RBPs belonging to the first quartile of most genomically-altered RBPs concerning tumor progression-related alterations (mRNA upregulation, genomic amplification, gene mutations or fusions); 2) All 398 RBPs presenting PPIs with well-known BC proteins (Supp. Table 6); 3) 160 RBPs having at least one immunohistochemical variation level towards protein overexpression (e.g. not detected to low); And 4) All 207 BC essential RBPs (Fig. 5A; Supp. Table 8-9).

We found five RBPs presenting the aforementioned tumor-associated characteristics: TFRC, KPNB1, PUF60, NSF, and SF3A3 (Fig. 6A). TFRC and KPNB1 have been previously implicated in BC⁴³⁻⁴⁶, while PUF60 has been associated with colon and non-small cell lung cancer^{47,48}. Interestingly, NSF and SF3A3 have never been studied in cancer. We also found 14 RBPs showing high genomic alterations, PPIs with BC proteins, and altered protein expression profiles in tumoral tissues. Although these proteins are not needed for tumor survival *ex vivo* (Supp. Table 8-9), they could be implicated in other tumorigenic processes; indeed, 11 of these RBPs have been described as BC tumor progressors⁴⁹⁻⁶⁰. Interestingly, PLEC has not been related with BC, but promotes migration and invasion of neck squamous cell carcinoma⁶¹. In addition, PRPF3 and MAGOHB have not been linked to cancer before.

Next, we focused on RBPs having putative tumor suppression profiles. In this concern, we compared our afore made analyses as follows: 1) 348 RBPs belonging to the first quartile of most genomically-altered RBPs concerning tumor suppression-related alterations (mRNA downregulation, deep deletions, gene mutations or fusions); 2) All 398 RBPs presenting PPIs with well-known BC proteins (Supp. Table 6); 332 RBPs having at least one immunohistochemical variation level towards protein downregulation (e.g. low to not detected); 4) All 207 BC essential RBPs (Fig. 5A; Supp. Table 8-9). Contrary to our previous

analysis, DEMETER2^{12,13} and CERES¹¹, which detect loss-of-function proteins, are now used to discriminate between suppressors and progressors RBPs. Thus, we identified 25 RBPs (Fig. 6B): 5 never studied in cancer (CSTF2T, SRRM1, CPSF7, EIF4G3, and ELOA), 6 associated with other cancer types (TNRC6B⁶², SEC31A⁶³, SPTAN1⁶⁴, MARK2⁶⁵, TNRC6A⁶⁶, and PMS1⁶⁷), 7 associated with BC (CASC3⁶⁸, HNRNPF⁶⁹, MYH9⁷⁰, TLN1⁷¹, DDX5⁷², TAF15⁷³, and EIF4G1⁷⁴), 3 already described as tumor suppressors in BC (CTNND1²³, LIMA1²⁴, AATF²⁵), and 4 identified as tumor suppressors in other cancer types (MEX3C⁷⁵, UPF1⁷⁶, CNOT1⁷⁷, and TNKS1BP1⁷⁸).

Discussion

Current oncological research generates large-scale datasets that contain undiscovered key aspects of tumor biology, and yet these databases are not fully exploited. *In silico* analyses of these data could therefore lead to the discovery of new cancer proteins^{29,79–81}. In this regard, RBPs are emerging as critical regulators of tumorigenesis¹⁷; however, they remain understudied in cancer research. In the present study, we analyzed and integrated RBPs genomic alterations, PPI networks, immunohistochemical profiles, and loss-of-function experiments to unravel new putative BC proteins.

The results of this research revealed for the first time that RBPs are equally altered than well-known BC proteins (Fig. 1A); this was expected since many RBPs are highly altered across cancer types²⁹ and have been *in silico* linked to cancer-related cellular processes⁸². Contrary to a predominant mRNA downregulation pattern across cancers²⁹, we found that most RBPs genomic alterations in BC are mRNA upregulation (68.7%) and amplification (15.4%) (Fig. 1B). This probably will increase RBPs cellular concentrations leading to dysfunctional post-transcriptional processes.

To determine how many RBPs have been previously studied in BC, we next analyzed the most recent catalog of cancer driver genes: NCG6³⁰. Only 14 RBPs have been catalogued as BC driver genes (Fig. 2A). This indicates that RBPs have been poorly investigated in breast carcinogenesis. Thus, to identify new putative BC RBPs, we first explored their genomic alteration profiles associated with tumor progression or suppression (Table 1, Supp. Table 2). As expected, we have found well-known BC progressors and suppressors proteins, such as DAP3¹⁸, MTDH¹⁹ or CCAR2²⁰, validating our strategy (Table 1). On the contrary, our strategy revealed RBPs that have not been previously associated with tumorigenesis, and yet they are highly altered in BC (e.g. TFB2M, C1ORF131 or DDX19A)

(Table 1). Interestingly, the most altered RBP (MRPL13) in our analysis has never been studied in cancer. MRPL13, along with other highly altered RBPs (Table 1), has only shown to interact with ESR2, a tumor suppressor in breast and other cancer types³³. This observation led us to investigate how many RBPs interact with ESR2; strikingly, we found that 30% of all RBPs interact with this receptor³³. ESR2 could probably exert its suppressive activity through post-transcriptional mechanisms implicating several RBPs; nevertheless, more research is needed to understand this observation.

Second, to further characterize RBPs associated with BC subtypes and staging, we hence analyzed RBPs genomic alterations (Fig. 2C-D). Interestingly, RBPs genomic alterations gradually increased from Normal to Basal subtype (Fig. 2C), i.e. from low to a high proliferative stage². Concordantly, metastasized tumors (stage IV) showed high frequencies of RBPs genomic alterations compared to non-metastasized samples (stage I to III) (Fig. 2D). Therefore, it seems that RBPs are acting as BC progressors rather than suppressors, which agrees with their genomic alteration profiles (Fig. 1B). This analysis also revealed highly altered RBPs per subtype or staging (Fig. 2C-D; Supp. Table 4-5) that could lead to the discovery of new clinical biomarkers or therapeutic targets. Indeed, SCAMP3 and HEATR6, which have not been studied in BC, presented similar degrees of genomic alterations (Fig. 2D) than well-known metastasis drivers: ARF1³⁴ and PARP1³⁵. In hepatocellular carcinoma cells, SCAMP3 knockdown has been shown to suppress cell proliferation⁸³, while HEATR6 has never been associated with tumorigenesis. Thus, more research is needed to understand their role in BC.

Interaction networks have been shown to be useful for identifying key tumoral proteins³⁷. In this regard, by analyzing PPIs between RBPs and well-known BC proteins, we identified SF3B1 and CDC5L at the core of two main networks (Fig. 3). While SF3B1 has been previously implicated in BC⁸⁴, CDC5L, which interacts with 14 BC proteins, has not been studied in this malignancy. However, CDC5L has been related with other cancer types, such as osteosarcoma⁸⁵ and prostate cancer⁸⁶, showing its possible role in breast carcinogenesis.

Next, we exploited the HPA database^{14–16} to identify differentially expressed RBPs in tumor breast tissues. We found 24 upregulated and 62 downregulated RBPs compared with normal tissues. As expected, our approach revealed well-known BC proteins. For instance, KPNA2 which has been known to enhance BC metastasis *ex vivo*²¹, is highly expressed in BC tissues (7 out of 12 tumor samples are classified as high) (Fig. 4B, Supp. Table 6). On the contrary, G3BP2 expression is reduced in tumoral breast tissues (Fig. 4B, Supp. Table

6); accordingly, loss of G3BP2 enhances tumor invasion and metastasis *in vivo*²². Interestingly, DARS2 which has never been related with BC is upregulated in our analysis (10 out 12 tumor samples are classified as high) (Fig. 4B, Supp. Table 6) and has been associated with hepatocarcinogenesis progression⁸⁷, showing its putative implication in BC. In addition, SUPT6H protein expression is diminished in breast tumoral tissues (Fig. 4B, Supp. Table 6) and has not been linked to this malignancy. Worthy of note, SUPT6H knockdown is associated with DNA damage via formation of RNA:DNA hybrids (R-loops) in HeLa cells⁸⁸, showing its possible role in breast tumorigenesis.

To identify essential RBPs for tumor survival, we next analyzed *ex vivo* loss-of-function screens: CERES¹¹ and DEMETER2^{12,13}. *In toto*, we identified 207 essential RBPs for tumor survival. This was expected since RBPs control every aspect of RNA metabolism. However, only 59 were characterized as essential by both computational methods (Fig. 5A; Supp. Table 8-9). Although CERES¹¹ and DEMETER2^{12,13} did not tested all human RBPs, therapeutic posttranscriptional BC research could therefore be focused on these 59 RBPs. However, more research is needed to deeply understand their carcinogenic roles. Also, we revealed essential RBPs per BC molecular subtype (Fig. 5B) that could be analyzed to better understand subtype-related posttranscriptional processes.

In extending the scope of our previous analyses, we finally reasoned that integration of all databases examined could narrow down the identification of potential BC RBPs. First, we focused on RBPs having putative tumor progression profiles unraveling 19 RBPs with tumorigenic characteristics according to our analyses (Fig. 5A). As expected, most of them (13 out 19) have been described as BC tumor progressors, controlling different cellular processes: migration, invasion, and metastasis^{43–46,49–60}. Interestingly, NSF, SF3A3, PRPF3, and MAGOHB have never been studied in cancer. While on the other hand, PUF60 has been associated with colon and non-small cell lung cancer^{47,48} and PLEC has been shown to promote migration and invasion of neck squamous cell carcinoma⁶¹. Next, we identified new RBPs having possible tumor suppression roles in BC. As discussed before and depicted in Fig. 1B and Fig. 2 C-D, RBPs seemed to act as progressors rather than suppressors. Although most of the proteins identified have been associated with breast and other cancer types, their suppressive roles remain undetermined^{62–74}. Only 3 have been described as tumor suppressors in BC (CTNND1²³, LIMA1²⁴, AATF²⁵), and 4 has been identified as tumor suppressors in other cancer types (MEX3C⁷⁵, UPF1⁷⁶, CNOT1⁷⁷, and

TNKS1BP1⁷⁸). Interestingly, 5 have never been studied in cancer: CSTF2T, SRRM1, CPSF7, EIF4G3, and ELOA.

In sum, individual and integrated analysis of the aforementioned databases led us to identify RBPs that have never been studied in BC but with defined tumorigenic functions in other cancer types. Thus, based on their tumorigenic characteristics presented in this study and their roles in other cancer types, we have identified new putative BC RBPs: 6 progressors (MRPL13, SCAMP3, CDC5L, DARS2, PUF60, and PLEC), and 5 suppressors (SUPT6H, MEX3C, UPF1, CNOT1, and TNKS1BP1). However, further research should focus on the mechanisms by which these proteins promote or suppress breast tumorigenesis, holding the promise of new therapeutic pathways along with novel drug development strategies.

Methods

Gene sets. A total of 1393 RBPs were extracted from Hentze *et al.*²⁷ and checked for new annotations using Ensembl (<http://www.ensembl.org>)^{89,90}. Only one duplicate was found: ENSG00000100101 and ENSG00000273899, both correspond to *NOL12*, leaving a final list of 1392 RBPs. BC genes (n = 171), related to Fig. 1A, were obtained from The Network of Cancer Genes 6.0 (NCG6)³⁰. Non-cancer gene list, related to Fig.1A, was constructed as follow: non-cancer genes from Piazza *et al.*³¹, without RBPs and NCG6³⁰ cancer genes, were reanalyzed using Piazza's OncoScore algorithm (<http://www.galseq.com/oncoscore.html>), giving a final list of 177 non-cancer genes (Supp. Table S1).

Genomic analysis. Genomic alterations of RBPs, non-cancer and BC genes were analyzed through the cBioPortal (<http://www.cbioportal.org/>)^{91,92} using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) database (n = 994 complete samples)⁵⁻¹⁰. To compare the aforementioned gene sets, genomic alterations per protein were corrected by the number of genes or individuals (Fig. 1A, Fig. 2C and D). A Mann–Whitney U test was used to compare genomic alterations between gene sets or clinical characteristics.

Network construction. Experimental and database interactions (Figure 3 and Table S3) between RBPs (n=1392) and BC proteins (n=171)³⁰, having an interaction score of 0.9 (highest confidence), were extracted from STRING database⁹³ and visualized using Cytoscape 3.7.1 platform⁹⁴.

Protein expression analysis. Immunohistological levels of 1212 available RBPs in normal and BC tissues were extracted from Protein Atlas version 18.1 (<https://www.proteinatlas.org/>)^{14–16}. Expression levels of normal tissues were taken from glandular cells, while a consensus level was manually generated for BC tissues (Table S6) based on tissue level frequency. Immunohistological images presented in Figure 4B were taken from <https://www.proteinatlas.org/ENSG00000182481-KPNA2/tissue/breast#img> (KPNA2 staining in normal tissue), <https://www.proteinatlas.org/ENSG00000182481-KPNA2/pathology/tissue/breast+cancer#img> (KPNA2 staining in tumoral tissue), <https://www.proteinatlas.org/ENSG00000138757-G3BP2/tissue/breast#img> (G3BP2 staining in normal tissue), <https://www.proteinatlas.org/ENSG00000138757-G3BP2/pathology/breast+cancer#img> (G3BP2 staining in tumoral tissue), <https://www.proteinatlas.org/ENSG00000109111-SUPT6H/tissue/breast#img> (SUPT6H staining in normal tissue), <https://www.proteinatlas.org/ENSG00000138757-G3BP2/pathology/breast+cancer#img> (SUPT6H staining in tumoral tissue),

Cancer dependency analysis. RBPs cancer dependency scores from CERES¹¹ (1288 available RBPs) and DEMETER2^{12,13} (1290 available RBPs) were obtained from the Dependency Map (DepMap) portal (<https://depmap.org/portal/>). Molecular subtypes of 82 (DEMETER2^{12,13}) and 28 (CERES¹¹) BC cell lines were obtained from Smith *et al.*⁴⁰, Dai *et al.*⁴¹, and Kao *et al.*⁴² (Supp. Table 10)

Table 1. Top ten most altered RBPs in Breast Invasive Carcinoma (TCGA, PanCancer Atlas)

Genomic alteration	Gene name	Number of alterations	Known BC molecular and cellular functions	Related to other cancer types	Pubmed number of citations
Amplification + mRNA upregulation + fusion + mutations	MRPL13	538	No. However, MRPL13 is a ESR2 protein interactor in MCF7 cells ³³	No	34
	DCAF13	535	Yes. DCAF13 has been shown to be overexpressed in 171 primary breast tumors ⁹⁵	Yes ⁹⁶	23
	YWHAZ	500	Yes. Often amplified in BC ⁹⁷ leading to increased glycolysis ⁹⁸ . YWHAZ is also a ESR2 protein interactor ³³	Yes	492
	TFB2M	460	No	No	36
	DAP3	457	Yes. DAP3 silencing contributes to breast carcinogenesis ¹⁸	Yes ⁹⁹	77
	NUCKS1	447	Yes. NUCKS1 has been shown to be overexpressed in breast tumors ¹⁰⁰	Yes ¹⁰¹	58
	MTDH	442	Yes. MTDH promotes cancer proliferation and metastasis ¹⁹	Yes ¹⁰²	273
	C1ORF131	432	No	No	13

	PTDSS1	430	No. However, PTDSS1 is a ESR2 protein interactor in MCF7 cells ³³	No	27
	RBM34	422	No. However, RBM34 is a ESR2 protein interactor in MCF7 cells ³³	No	35
Deep deletion + mRNA downregulation + fusion + mutations	CCAR2	367	Yes. CCAR2 functions as a tumor suppressor ²⁰	Yes ¹⁰³	149
	DDX19A	225	No	No	24
	DHX38	166	No. However, DHX38 is a ESR2 protein interactor in MCF7 cells ³³	No	50
	ADD1	160	No. However, ADD1 is a ESR2 protein interactor in MCF7 cells ³³	Yes ¹⁰⁴	223
	ZC3H18	122	No. However, ZC3H18 is a ESR2 protein interactor in MCF7 cells ³³	No	39
	NCBP3	121	No. However, NCBP3 is a ESR2 protein interactor in MCF7 cells ³³	No	26
	KMT2C	119	Yes. KMT2C regulates ER α activity ¹⁰⁵	Yes, tumor suppressor in esophageal squamous cell carcinoma ¹⁰⁶	88
	RARS2	118	No	No	26
	EIF4ENIF1	115	No	No	52
	NMT1	103	No	Yes ¹⁰⁷	92

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Acknowledgements

A.I. acknowledges the Spanish Ministry (MEIC) to EMBL partnership, Severo Ochoa and the CERCA program.

Figure legends

Figure 1. Genomic alterations of RNA-binding proteins (RBPs) in BC. (A) Frequency of genomic alterations per gene set (non-cancer genes [n=170], BC genes [n=171], and RBPs [n=1392]) using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas)^{5–10} database. Genomic alterations per patient were corrected by the number of genes; a Mann–Whitney U test was used to compare genomic alterations between gene sets. ** = highly significant; ns = not significant. (B) A pie chart describing RBPs genomic alterations types.

Figure 2. Identification of Highly Altered BC RBPs. (A) An histogram describing the status of RBPs in the Network of Cancer Genes 6.0 (NCG6). (B) A Venn diagram depicting the relationship between RBPs and the estrogen receptor 2 (ESR2) protein interactome. RBPs genomic alterations per subtype (C) and stage (D) using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas)^{5–10} database are also displayed.

Figure 3. Experimental PPIs between RBPs and well-known BC proteins. An interaction network, constructed using STRING database and Cytoscape 3.7.1 platform, is presented; red, BC proteins; green, RBPs.

Figure 4. Immunohistochemical protein expression profile of RBPs between healthy and breast tumor tissues. (A) A correlation plot, comparing RBPs immunohistochemical levels between normal and BC tissues, is presented. Circle sizes correlate with the number of RBPs presented in each intersection. (B) Representative immunohistochemical stains of four RBPs (upregulated in red: KPNA2 and DARS2; downregulated in blue: G3BP2 and SUPT6H) on normal and tumor breast tissues according to The Human Protein Atlas (HPA).

Figure 5. RBPs BC dependencies. (A) The distribution of dependency scores of 1290 (DEMETER2) and 1288 RBPs (CERES) is shown. (B) A Venn diagram comparing BC essential RBPs per subtype is presented.

Figure 6. Unraveling putative BC RBPs. (A) A Venn diagram depicting the number of unique and shared RBPs across the four cancer progression profiles. (B) A Venn diagram showing the number of unique and shared RBPs across the four cancer suppression profiles.

A

B

A

B

C

D

A

B

A

B

A

Putative progressors

DAP3
PARP1
CTTN
CACYPB
ARHGEF2
PRPF3
NDRG1
KPNA2
PLEC
UBE2I
RBBP6
MAGOHB
HDAC2
DKC1

PUF60
TFRC
KPNB1
NSF
SF3A3

B

Putative suppressors

CSTF2T PMS1
SRRM1 CASC3
CPSF7 HNRNPF
EIF4G3 MYH9
ELOA TLN1
TNRC6B DDX5
SEC31A TAF15
SPTAN1 EIF4G1
MARK2 CTNND1
TNRC6A LIMA1
AATF CNOT1
MEX3C TNKS1BP1
UPF1

Tumor progression related genomic alterations (TCGA)

Protein changes (HPA)

Essential RBPs (DepMap)

Tumor suppression related genomic alterations (TCGA)

BC protein interactors (STRING)