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Expression of CDK8 and CDK8-interacting Genes as Potential Biomarkers in Breast Cancer

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Abstract

CDK8 and its paralog CDK19, in complex with CCNC, MED12 and MED13, are transcriptional regulators that mediate several carcinogenic pathways and the chemotherapy-induced tumor-supporting paracrine network. Following up on our previous observation that CDK8, CDK19 and CCNC RNA expression is associated with shorter relapse-free survival (RFS) in breast cancer, we now found by immunohistochemical analysis that CDK8/19 protein is overexpressed in invasive ductal carcinomas relative to non-malignant mammary tissues. Meta-analysis of transcriptomic data revealed that higher CDK8 expression is associated with shorter RFS in all molecular subtypes of breast cancer. These correlations were much stronger in patients who underwent systemic adjuvant therapy, suggesting that CDK8 impacts the failure of systemic therapy. The same associations were found for CDK19, CCNC and MED13. In contrast, MED12 showed the opposite association with a longer RFS. The expression levels of CDK8 in breast cancer samples were directly correlated with the expression of MYC, as well as CDK19, CCNC and MED13 but inversely correlated with MED12. CDK8, CDK19 and CCNC expression was strongly increased and MED12 expression was decreased in tumors with mutant p53. Gene amplification is the most frequent type of genetic alterations of CDK8, CDK19, CCNC and MED13 in breast cancers (9.7% of which have amplified MED13), whereas point mutations are more common in MED12. These results suggest that the expression of CDK8 and its interactive genes has a profound impact on the response to adjuvant therapy in breast cancer in accordance with the role of CDK8 in chemotherapy-induced tumor-supporting paracrine activities.

Keywords

Breast cancer; CDK8; CDK19; Cyclin C; MED12; MED13; microarray data mining; tissue microarrays

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BREAST CANCER BIOMARKERS GUIDE THE CHOICE OF THERAPY

Breast cancer remains a major health challenge, with 234,190 estimated new cases in the US in 2015 and 40,730 expected to die from the disease [1]. Several biomarkers have long been used to guide the choice of therapy in breast cancer. In particular, expression of estrogen receptor and progesterone receptor is used as an indication for hormone therapy, whereas HER2/Neu overexpression indicates the use of HER2-targeted agents. Research on new biomarkers for early detection and prediction of post-surgical recurrence and treatment response in breast cancer is rapidly expanding [2]. This research has received a great boost from the accumulation of transcriptomic data in breast cancer and the powerful bioinformatics methods for its processing, which, after validation, resulted in the development of RNA-based multigene tests to predict breast cancer recurrence. These tests, such as Oncotype DX and MammaPrint were found to have a positive impact on the selection of adjuvant therapy [2]. Several potential biomarkers are being actively investigated for the prediction of systemic therapy response, such as polymorphic variants or mutations in CYP2D6 or PIK3CA and the expression of RARA, STAT3, Lin28 and several microRNAs but none of these biomarkers have yet been verified for diagnostic use [2]. There is continuous need to identify additional biomarkers that can be added to multigene tests in order to guide therapeutic choices more precisely. In the present study, we have investigated the expression of genes of the transcription-regulatory CDK8 complex, recently identified as a pleiotropic mediator of carcinogenesis and chemotherapy failure, as potential new biomarkers of prognosis and therapy response in breast cancer.

CDK8/19: TRANSCRIPTION-REGULATING ONCOGENIC KINASES

Cyclin-dependent kinase 8 (CDK8) and its closely related paralog CDK19 (80% identity) are transcription-regulating serine/threonine kinases that, unlike better-known members of the CDK family, such as CDK1 (CDC2), CDK2 or CDK4/6, do not play a general role in cell cycle progression [3]; CDK8 depletion does not inhibit the growth of normal cells [4]. CDK19 is not expressed as universally as CDK8 but transcriptional effects of CDK8 and CDK19 have been shown to be largely overlapping (albeit non-identical) [5]. CDK8 and CDK19 are alternative subunits of the regulatory CDK module of the Mediator complex that links transcription-initiating factors with RNA Polymerase II (Pol II). The other components of the CDK module are Cyclin C (CCNC) (the regulatory subunit of CDK8/19 kinase), MED12 and MED13 [3]. The CDK module was at first implicated in the inhibition of transcription [6–8], and this repressive effect was reported in some systems to be independent of the kinase activity of CDK8 [7]. On the other hand, recent studies have identified multiple roles of CDK8 as a positive regulator of transcription initiated by various signals [3]. The CDK module is capable of phosphorylating the C-terminal domain (CTD) of Pol II, which enables the elongation of transcription [9,10]. CDK8, however, is required for Pol II CTD phosphorylation only in a context-specific manner, at silent genes when they become activated by transcription-initiating signals, as demonstrated for genes induced by serum [9] or HIF1A [10]. As a result of this selective activity, CDK8/19 inhibition has little effect on most cell types under homeostatic conditions [11] but it prevents transcriptional reprogramming triggered by various signals [9,10].

CDK8/19-mediated transcriptional reprogramming is especially pertinent in cancer, where CDK8 was identified as a positive transcriptional regulator in cancer-relevant signaling pathways, including Wnt/β-catenin [12], the serum response network [9], the TGFβ signaling pathway [13] and HIF1A-mediated response to hypoxia [10,14]. CDK8 has been identified as an oncogene, which is amplified in a substantial fraction of colon cancers [12]. CDK8 has also been implicated in melanomagenesis [15], associated with the cancer stem cell phenotype [16] and with shorter survival in colorectal cancers [17]. Our work has identified CDK8 as a mediator of damage-induced tumor-promoting paracrine activities of both tumor and normal cells; as a consequence, CDK8/19 inhibition increases the efficacy of chemotherapy [11]. Although the oncogenic role of CDK8 has been firmly established, a recent report has identified its binding partner CCNC as a haploinsufficient tumor suppressor in T-cell leukemia [18]. The latter effect of CCNC has been linked to the phosphorylation of Notch1 [18], which in its turn is known to possess a mixture of tumor-suppressive and tumor-promoting activities [19].

In our previous study, using the KM-plotter online survival analysis tool [20], we have analyzed survival associations of the expression of CDK8, CDK19 and CCNC in a compilation of Affymetrix microarray gene expression data and found that higher CDK8, CDK19 and CCNC expression was correlated with shorter Relapse Free Survival (RFS) in breast and ovarian cancers, with the strongest association observed in breast cancers [11]. We have now extended this analysis by analyzing CDK8/19 protein expression in benign and malignant breast tissues and by extensive analysis of genomic and transcriptomic data on CDK8, CDK19 and their binding partners CCNC, MED12 and MED13 in breast cancer.

IMMUNOHISTOCHEMICAL ANALYSIS OF CDK8/19 PROTEIN EXPRESSION IN BENIGN AND MALIGNANT BREAST TISSUES

Since the paracrine effects of CDK8/19 are exerted in both tumor and normal cells, the microarray data [11] obtained from bulk tissue samples could reflect CDK8/19 expression either in the tumor or in the stromal cells that are inevitably present in breast cancer samples. To investigate CDK8/19 expression *in situ* in normal, pre-neoplastic and malignant breast tissues, we have carried out immunohistochemistry (IHC) analysis of tissue arrays from formalin-fixed, paraffin-embedded patient breast biopsies obtained from US Biomax, Inc. (BR-243F, BR-952, BR-954, BR-1003, BR-1006, BR-1503, BR-2082, BR-6161, BR-10010). Following washing, epitope unmasking, and peroxidase blocking, the arrays were incubated overnight at 4°C with the primary antibody against CDK8 (Santa Cruz sc-1521, goat polyclonal, 1:250 dilution) using Antibody Amplifier (ProHisto, LLC). This sc-1521 antibody reacts not only with CDK8 but also with CDK19 [5]. Polymer-based anti-goat secondary antibody (EnVision System-HRP kit, DakoCytomation) was used according to manufacturer's protocol. For antigen detection, all slides were incubated for exactly the same length of time with chromogenic substrate DAB (3,3'-Diaminobenzidine) and counterstained with methyl green. To evaluate antigen expression, double-blinded semi-quantitative ImmunoReactivity Scoring (IRS) was performed microscopically with 10 × and 40 × objectives in accordance with the German Immunohistochemical Scoring System [21] by two independent observers, with satisfactory concordance. Some patients' samples were

repeated on more than one tumor array and the scores for repeating samples were averaged in the final scoring pool. A total of 496 normal, hyperplastic, benign and malignant breast samples were scored.

Fig. **1a–f** shows representative images of CDK8/19 staining in normal, hyperplastic, benign and cancerous mammary tissues. The IHC scores for epithelial cell staining in all the samples are compiled in Fig. **1g**. The significance of differences between the IRS values of different categories of tissues was obtained by the chi square test. This analysis showed that the average CDK8/19 staining intensity is significantly higher in primary invasive ductal carcinomas (IDC) than in other types of breast cancer and in benign, hyperplastic or normal mammary tissues. The intensity of CDK8/19 staining varied among the IDC samples (Fig. **1d–f**). The strongest CDK8/19 staining was observed in IDC tumor cells although stromal cells also stained positively for CDK8/19 (Fig. **1d–f**). Based on this analysis, we conclude that CDK8/19 protein expression is elevated in IDC, the predominant type of breast cancer, and that tumor cells are likely to constitute the principal source of CDK8 mRNA in breast cancer microarrays.

The finding of elevated CDK8/19 expression in IDC relative to normal mammary tissues suggest a possible oncogenic role of CDK8 and its interacting genes in breast cancer. Indeed, Xu *et al*. [22] very recently reported that CDK8 is implicated in breast carcinogenesis and that the expression of CDK8 protein (or, more precisely, CDK8/19, since these authors used the same cross-reactive antibody as in our study) is positively correlated with tumor status, nodal metastasis and stage in breast cancer. CDK8 gene expression was found to be elevated by Skp2 SCF complex that promotes the ubiquitination and degradation of histone variant macroH2A, a negative regulator of CDK8 expression [15]. The oncogenic role of CDK8 in breast cancer was suggested to be due to CDK8 facilitating the degradation of cell cycle inhibitor p27 [22].

GENETIC ALTERATIONS OF CDK8 GENE COMPLEX IN BREAST CANCERS

CDK8 and CDK19 do not function in isolation but require CCNC binding for their kinase activity; CDK8/19-CCNC complex is also associated with MED12 and MED13 in the CDK module of the Mediator. We have queried genetic alterations of CDK8, CDK19, CCNC, MED12 and MED13 in the TCGA sequence data of 968 breast cancers using cBio Cancer Genomics Portal [23, 24]. As shown in Fig. **1H**, the most frequently altered gene in this set is MED13 (located on chr 17q23 according to Ensembl), which is altered in 11% of breast cancers, with gene amplification being by far the most common change (94/968 or 9.7%). This exceptionally high rate of gene amplification was associated with higher MED13 expression at the RNA level in samples with MED13 gene amplification ($p = 1.1E-09$, RNA-seq data). Gene amplification was also the most frequent alteration of CDK8 (chr 13q12), CDK19 (chr 6q21) and CCNC (chr 6q16.2), with 5 of 14 CDK8-amplified cases also showing MED13 amplification, and 10 of 21 CDK19-amplified cancers also amplifying CCNC. In contrast to the other members of the CDK module, the most common alterations of MED12 (chr Xq13) were point mutations.

EXPRESSION OF CDK8 AND ITS INTERACTIVE GENES IS ASSOCIATED WITH SHORTER RELAPSE-FREE SURVIVAL AND FAILURE OF ADJUVANT SYSTEMIC THERAPY IN DIFFERENT BREAST CANCER SUBTYPES

We have extended our previous meta-analysis of Affymetrix microarray data for survival correlations of CDK8 expression [11]. Breast cancer datasets were identified in GEO (http://www.ncbi.nlm.nih.gov/gds) using the GEO platform IDs "GPL96", "GPL570", "GPL571", "GPL6947" and "GPL4133" and the keywords "breast", "cancer" and "survival". The following datasets were used: GSE1456, GSE2034, GSE2990, GSE3494, GSE4922, GSE6532, GSE7390, GSE11121, GSE12093, GSE5327, GSE9195, GSE16391, GSE12276, GSE2603, GSE17705, GSE21653, GSE16446, GSE17907, GSE19615, GSE20685, GSE20711, GSE26971, GSE31448, GSE31519, E-MTAB-365, GSE20194, GSE20271, GSE32646, GSE18728, GSE23988, GSE41998, GSE16716, E-TABM-43, GSE25066, GSE42568, GSE45255, GSE37946, GSE4611, GSE46184, GSE22093. The database was constructed as described previously [18]. Only datasets including at least 30 patients were considered, all together 3,491 breast cancer patients with Affymetrix HGU array data were processed. The average relapse-free survival of these patients is 6.3 years, 77% of the patients are ER positive and 31% are lymph node positive. 2,015 patients were known to receive systemic therapy after sample collection for microarray analysis and 1,000 received no systemic therapy.

We analyzed the correlations of CDK8 expression (Affymetrix dataset 204831_at) with RFS in all breast cancers and in their major molecular subtypes: luminal A, luminal B, basal and HER2+ using KM-plotter online survival analysis tool [25] as described previously [26]. Kaplan-Meier plots in Fig. **2** show that higher CDK8 expression was significantly associated with shorter RFS in all cancers and in each of the four subtypes. This correlation was validated in an independent TCGA RNA-Seq dataset (see below) and by the recent study of Xu *et al*. [22] who found that CDK8/19 protein expression significantly predicted disease-specific and metastasis-free survival.

We have also analyzed survival associations in the subsets of systemically untreated patients and systemically treated patients in all cancers and patients who were exposed to adjuvant systemic therapy after surgery (when the samples were collected for microarray analysis) within each of the molecular subtypes (except for the smallest HER2+ dataset). The RFS correlations of CDK8 expression were very different between the treated and the untreated patients. Higher CDK8 was weakly associated with shorter RFS among the untreated cancers in the luminal A category and showed no significant association for the untreated luminal B and basal cancers. In contrast, the CDK8/RFS association was very strong for the systemically treated categories of each subtype (Fig. **2**), suggesting that higher CDK8 expression is associated primarily with the failure of systemic adjuvant therapy rather than with tumor relapse that would result from treatment-unrelated phenotypes. Higher CDK8 expression was associated with shorter survival of all breast cancer patients in respect not only to RFS but also to Distant Metastasis Free Survival (DMFS) (HR=1.51, p=9.9E-05) and, to a lesser extent, to Overall Survival (OS) (HR=1.41, p=0.0044). The preferential association of CDK8 with the survival of treated rather than untreated breast cancer patients

was also obvious for DMFS (HR=1.43, p=0.036 for untreated and HR=2.01, p=2.5e-05 for treated patients) but was not observed in the smaller datasets that were available for OS (data not shown).

The above observations suggest that CDK8 expression *per se* plays little role in the relapse of luminal B and basal cancers in the absence of treatment but it is strongly associated with the failure of systemic therapy. Since these cancers are largely treated with conventional chemotherapeutic drugs, the observed association dovetails with our previous report that CDK8 is a key mediator of chemotherapy-induced tumor-promoting paracrine activities and that CDK8 inhibition sensitizes tumors to chemotherapy [11]. On the other hand, the associations observed in the estrogen receptor (ER) positive tumors of the most common luminal A subtype cannot be so readily explained by the previous data. Such tumors typically receive endocrine therapy, with or without chemotherapy, and the strong correlation between CDK8 expression and the therapy failure observed in this subtype would suggest a possible role for CDK8 in the response to endocrine therapy. In addition, the finding that CDK8 is associated with shorter RFS even in the untreated luminal A cancers (but not in the other subtypes) also suggests a functional link between CDK8 and ER. Based on these findings, we are conducting experimental analysis of the possible role of CDK8 in the ER transcriptional activity.

One could expect that CCNC and possibly also CDK19, MED12 and MED13 could display prognostic associations that would be similar to those observed for CDK8. We have analyzed the survival impact of these genes using the following Affymetrix probesets selected using JetSet [27]: CCNC, 201955_at; MED12, 216071_x_at; CDK19 (CDC2L6), 212899_at; MED13, 201987_at; TOP2A, 201292_at; MYC, 202431_s_at. Fig. **2b** shows Kaplan-Meier plot analysis of CDK19, CCNC, MED13 and MED12 in all the breast cancer patients as well as in the treated and the untreated patient datasets. The results for CDK19, CCNC and MED13 (Fig. **3**) are remarkably similar to those observed for CDK8 (Fig. **2**), with higher expression of these genes associated with shorter RFS among all breast cancers (including the individual molecular subtypes, not shown) and a much stronger association among the treated than among the untreated patients. The concurrence among different members of CDK8/19-CCNC complex confirms the association with treatment failure observed for CDK8. Surprisingly, MED12 expression showed the opposite prognostic association, with higher MED12 associated with longer RFS, both in the treated and in the untreated patients (Fig. **3**).

It is instructive to compare the above RFS associations obtained for CDK8 and its interactive genes, which are not directly involved in cell proliferation, with the correlations observed for TOP2A, a proliferation marker expressed primarily in cycling cells [28]. Such cell-proliferation genes comprise a major component of multigene lists used for RNA-based breast cancer diagnostics, such as MammaPrint [29]. TOP2A, in particular, was found in an earlier analysis to be a very strong negative predictor of RFS in the total set of breast cancers [20]. As shown in Fig. **3**, higher TOP2A is strongly associated with lower RFS in all breast cancers but in contrast to CDK8, CDK19, CCNC or MED13, this association was independent of treatment, suggesting that higher proliferation is a treatment-independent negative prognostic marker. We also noted that higher TOP2A expression was associated

with shorter RFS in luminal A (HR=2.24, p<1E-16) and luminal B subtypes (HR=1.79, p=1.6E-06), but TOP2A shows no significant survival associations in basal cancers, whereas higher TOP2A shows the opposite association with longer RFS in the HER2+ subtype (HR=0.54, p=0.0035). Hence, the prognostic correlations of CDK8 and its interactive genes in breast cancer are drastically different from those that are observed with a proliferation-specific gene.

The correlations between the expression of CDK8 and its interactive genes with negative prognosis in breast cancer were validated through the analysis of the TCGA RNA-Seq dataset of 618 patients. RNA-seq data for breast cancer samples [30] were published in The Cancer Genome Atlas (TCGA) of the National Cancer Institute (http://cancergenome.nih.gov/). We have downloaded the pre-processed level 3 data generated by the Illumina HiSeq 2000 RNA Sequencing Version 2 platform. For each of these samples, gene expression was determined using a combination of MapSplice and RSEM. The individual patient files were combined in R using the plyr package [31]. Using the same analysis as for the Affymetrix dataset, we found that shorter RFS was associated with higher expression of CDK19 (HR=3.2, p=0.0051), CDK8 (HR=2.0, p=0.017) and CCNC (HR=2.2, p=0.04), but no significant correlations were observed for MED12 and MED13 in this dataset.

CORRELATIONS OF CDK8 EXPRESSION IN BREAST CANCERS WITH ITS INTERACTIVE GENES AND MYC

We have asked if the observed similar and dissimilar RFS associations for CDK8 versus its interactive genes could reflect positive or negative correlations of the expression levels of these genes among breast cancer samples. To simplify the correlation analysis, all 3,491 samples were arranged by their CDK8 signal values and separated into 35 bins of 100 tumors (91 tumors in the last bin). The median CDK8 expression levels for these 35 bins were plotted against the median expression levels of CDK19, CCNC, MED12 and MED13 (Fig. **4a**). This analysis revealed striking positive correlations between the expression levels of CDK8 and those of CDK19, CCNC and MED13 but a negative correlation with MED12 expression (Fig. **4a**). The corresponding R2 correlations for the 35 bins and the P-values (determined for all 3,491 samples by Spearman rank correlation) are shown in Fig. **4a**. The positive correlations between the expression of CDK8, CDK19 and their interactive proteins CCNC and MED13 suggest the existence of common regulatory mechanisms or common selection pressures that define the expression of these genes in cancers. CDK8, CDK19, CCNC and MED13 also share the same association with RFS in breast cancers: a very strong association with shorter RFS in systemically treated but not in the untreated patients (Figs **2** and **3**). This common predictive pattern between CDK8, its kinase isoform and their interactive proteins provides a strong independent confirmation of the association between CDK8 expression and the failure of adjuvant therapy.

MYC oncogene is frequently amplified or overexpressed in breast cancers and MYC amplification is significantly correlated with aggressive tumor phenotypes and poor clinical outcomes [32]. CDK8 was shown to regulate MYC protein and downstream MYC target gene expression in colon cancer [16], and we have asked if CDK8 expression in breast

cancers would correlate with the expression of MYC. CDK8 expression in breast cancers showed a very strong positive correlation with MYC expression, when the tumor samples were distributed into bins according to the expression of either CDK8 or MYC (Fig. **4a**), suggesting that CDK8 may also regulate MYC in breast cancers.

ELEVATED EXPRESSION OF CDK8, CDK19, CCNC AND MYC IS ASSOCIATED WITH P53 MUTATIONS

We have also investigated if the expression of CDK8 and its interactive genes correlates with the p53 mutation status, using Affymetrix microarray gene expression data in 319 patients with known p53 status, as described [33]. The results, presented in Fig. **4b**, show that p53 mutations were associated with a striking increase in the expression of CDK8, CDK19 and CCNC and with a decreased MED12 expression, whereas MED13 expression was unaffected by the p53 status. Importantly, MYC expression is known to be upregulated by p53 mutations [34], and our analysis in breast cancers (Fig. **4b**) confirmed these observations. These findings raise a question if p53 mutations may regulate the expression of CDK8 and its interactive proteins, just as K-RAS mutations activate CDK8 expression in pancreatic cancers [35].

CDK8-MED12 DICHOTOMY IN BREAST CANCER

The above-described analyses provided a surprising conclusion that the two Mediator subunits that interact with CDK8/19-CCNC in the CDK module of the Mediator showed the opposite correlations with CDK8 expression and survival. While MED13 expression was positively correlated with that of CDK8, CDK19 and CCNC, MED12 expression was negatively correlated with CDK8 expression and MED12, in contrast to CDK8, CDK19, CCNC and MED13 was associated with positive prognosis in breast cancers. In addition, the p53 mutant status was associated with lower MED12 expression, in contrast to the higher expression of CDK8, CDK19 and CCNC. Furthermore, the most common type of genetic changes associated with MED12 in breast cancer is point mutations rather than gene amplification as for the other members of the CDK module.

Whereas MED13 links the CDK module with the rest of Mediator, MED12 was shown to stimulate the CDK8 kinase activity within the CDK module [36]. On the other hand, MED12, in contrast to CDK8, is found not only in the nucleus but also in the cytoplasm, where MED12 was shown to interact with TGFβR2 and to inhibit the TGFβ pathway, an activity independent of the role of the nuclear MED12 in the transcriptional Mediator complex [37]. Furthermore, while MED12 displays tumor-promoting activities in prostate cancer [38], it undergoes loss-of-function mutations in uterine leiomyomas [39]. Remarkably, the same MED12 mutations were also found and characterized in breast fibroadenomas [40] and benign phyllodes tumors of the breast [41, 42] but in the case of the fibroadenomas these mutations (which are different from those found in the TCGA breast cancer panel) occurred not in the epithelial mammary cells but rather in the stromal tissue, where they were associated with dysregulated estrogen signaling and extracellular matrix organization [40]. In light of these findings, one should consider the possibility that higher

MED12 expression that we found to be associated with positive prognosis in breast cancer microarray samples may reflect the stromal cell contribution.

CONCLUSIONS

In summary, the present analysis demonstrates that the previously observed [11] strong association between higher expression of CDK8, CDK19 and CCNC with shorter RFS in breast cancer is due primarily to the association of the CDK8 complex with the failure of adjuvant therapy, which should be due at least in part to the role of this transcription regulatory complex in chemotherapy-induced tumor-promoting paracrine activities [11]. The results of our analysis warrant further investigation of the validity of CDK8 and its interacting genes as a new class of cancer biomarkers that may predict relapse-free survival after systemic therapy.

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CONFLICT OF INTEREST

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Biography

E.V. Broude

LIST OF ABBREVIATIONS

CCNC	cyclin C
CDK19	cyclin-dependent kinase 19
CDK8	cyclin-dependent kinase 8
CTD	C-terminal domain
CYP2D6	cytochrome P450 2D6
DMFS	distant metastasis free survival
ER	estrogen receptor

GEO	Gene Expression Omnibus
HR	hazard ratio
IDC	invasive ductal carcinomas
IHC	immunohistochemistry
KM	Kaplan-Meier
OS	overall survival
PIK3CA	Phosphatidylinositol-4,5-biphosphonate 3-kinase
Pol II	RNA polymerase II
RARA	Retinoic acid receptor alpha
RFS	relapse-free survival
STAT3	signal transducer and activator of transcription 3
TCGA	The Cancer Genome Atlas
TGFβ	transforming growth factor beta
TGFβR2	receptor 2 of transforming growth factor beta
TIMP-1	tissue inhibitor of metalloproteinase 1
TOP2A	topoisomerase 2 alpha

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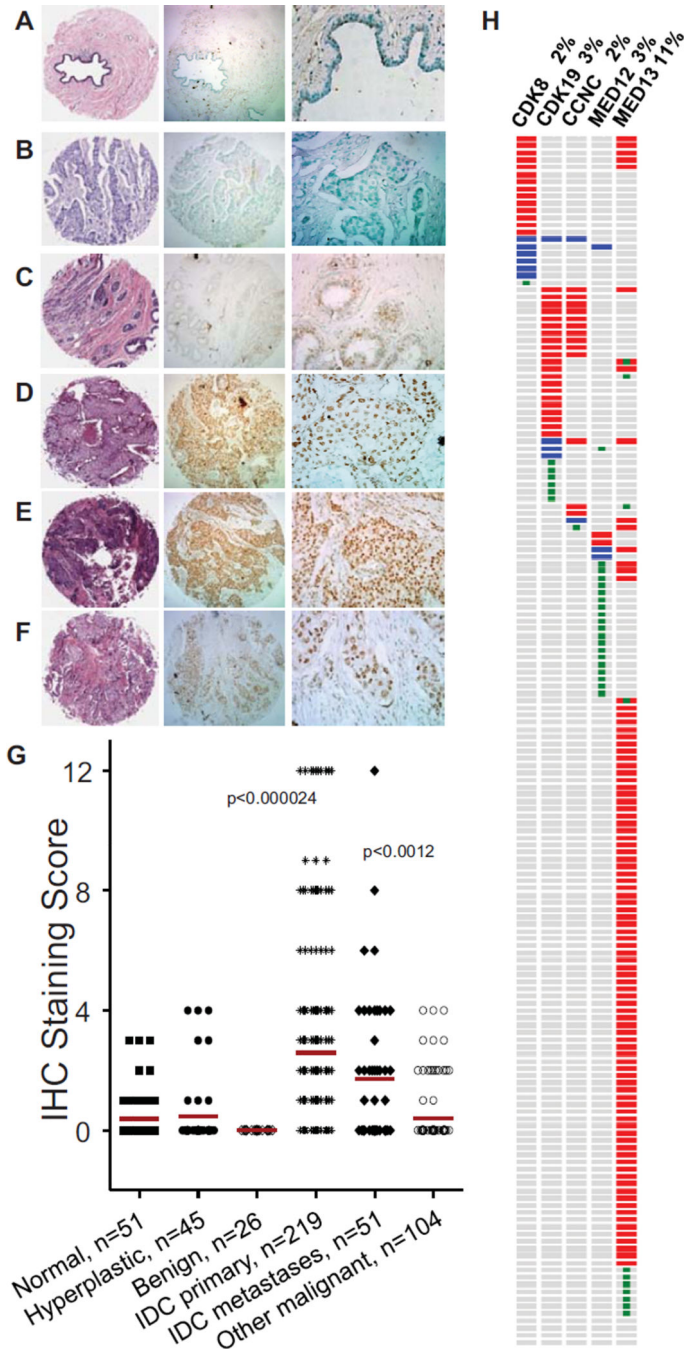

Fig. (1).

CDK8/19 protein expression and genetic alterations of CDK module components in breast cancers. A–F: representative images of CDK8/19 IHC staining in different types of mammary tissues. Left: H&E staining, 10 × objective. Center: CDK8/19 IHC (DAB) with methyl green counterstain, 10 × objective, Right: same as center, 40 × objective. A. Normal breast tissue; B. Epithelial hyperplasia; C. Lobular carcinoma; D–F. Infiltrating ductal carcinoma, not otherwise specified (3 different tumors); G: IHC scores for different types of mammary tissue samples. H: genetic alterations of CDK8, CDK19, CCNC, MED12 and

MED13 in TCGA panel of 968 breast cancers; only cancers showing alteration in any of these genes are presented. Numbers indicate the combined frequency of all genetic alterations for each gene. Red: gene amplification. Blue: homozygous deletion. Green: point mutation.

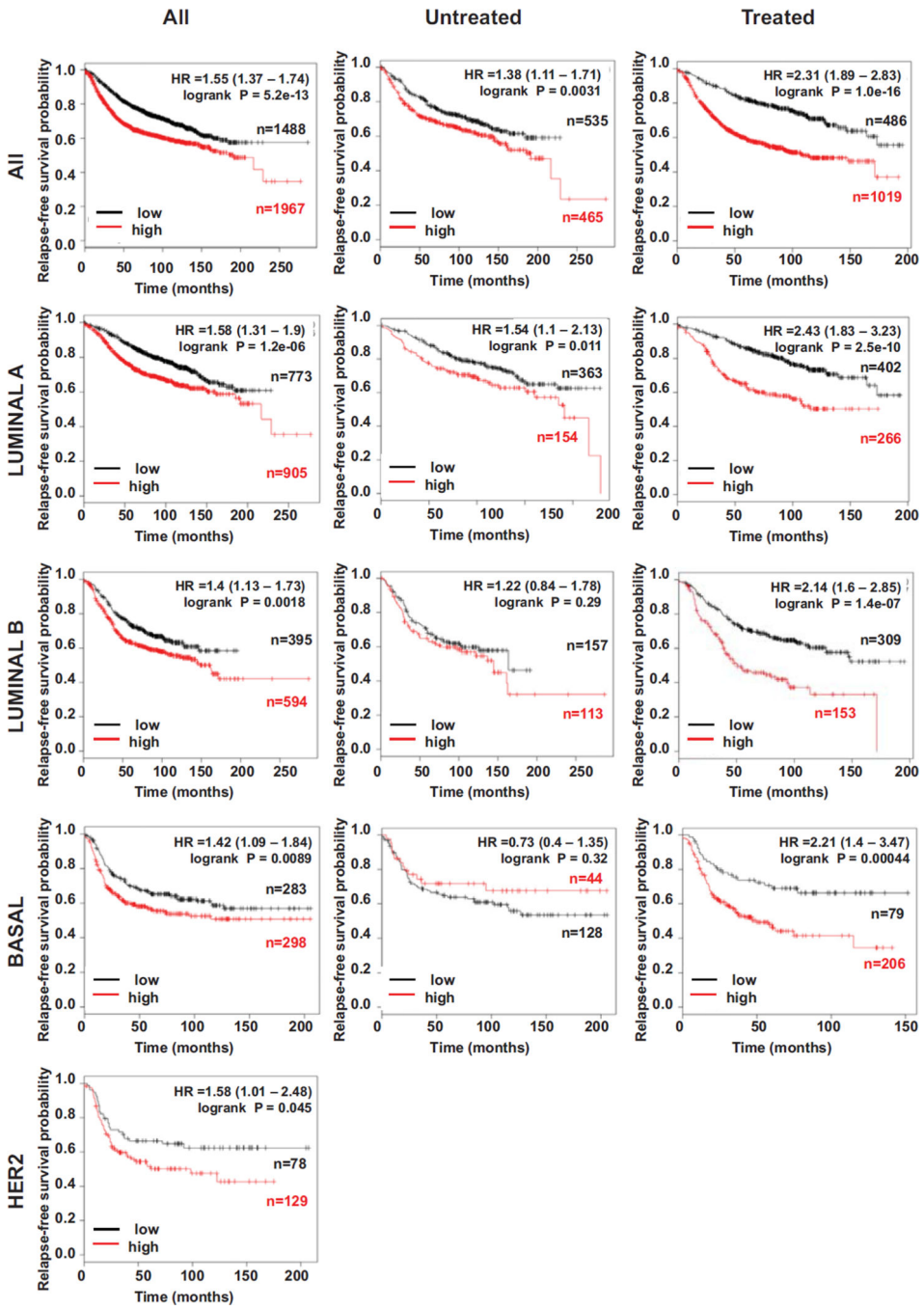

Fig. (2).

Association of CDK8 expression with RFS in Affymetrix breast cancer microarray data determined using KM-plotter online survival analysis tool (http://kmplot.com/analysis/) with "Auto select best cutoff" option. The results are shown for all breast cancers and their molecular subtypes, including subsets that did not receive or received systemic therapy after sample collection. Note that the sums of the patient numbers in individual subtypes are less than the number of all patients, since the molecular subtype or the treatment status was not reported for every patient.

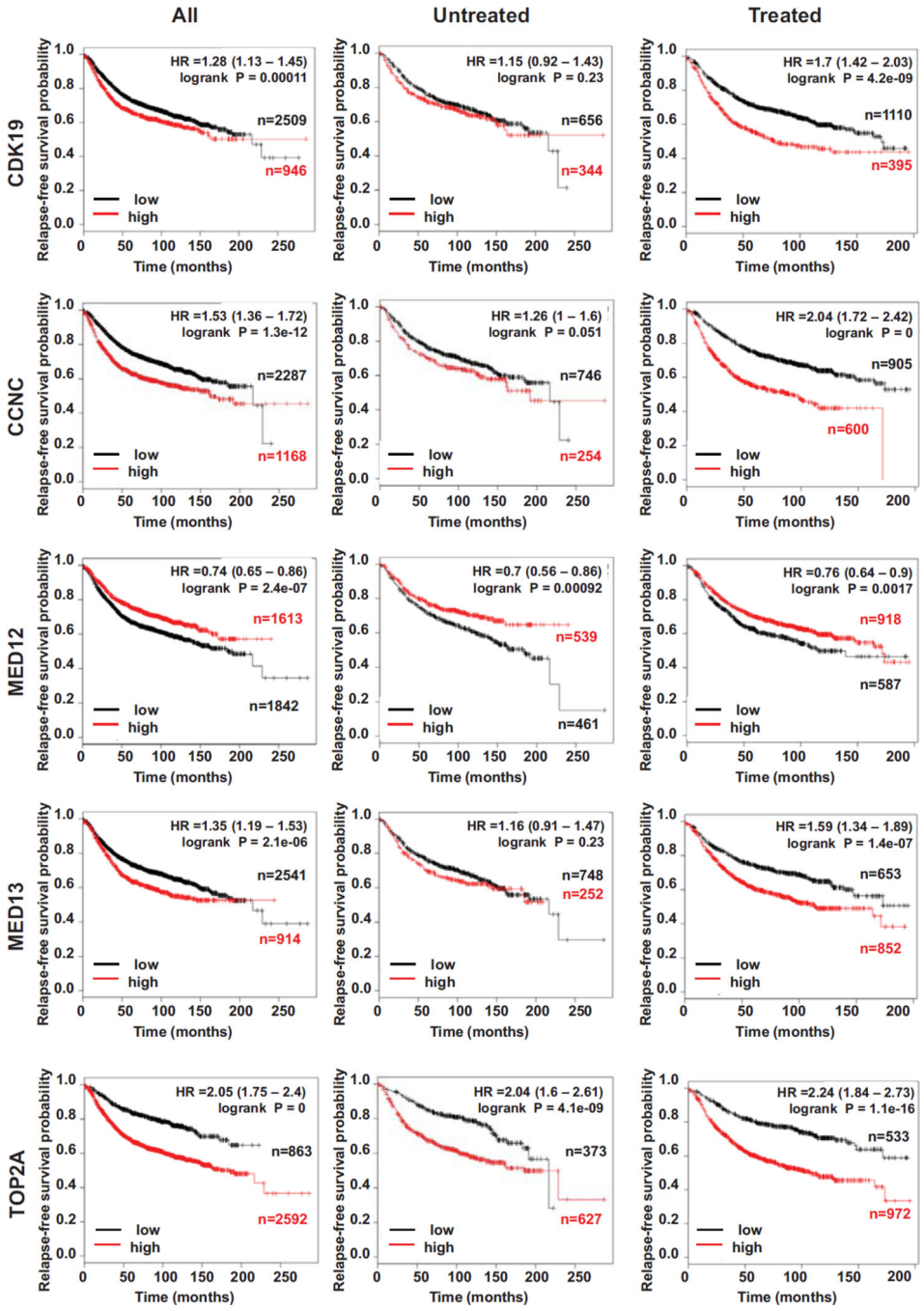

Fig. (3).

Association of CDK19, CCNC, MED12, MED13 and TOP2A expression with RFS in Affymetrix breast cancer microarray data, plotted for all patients and the subsets that did not receive or received systemic therapy.

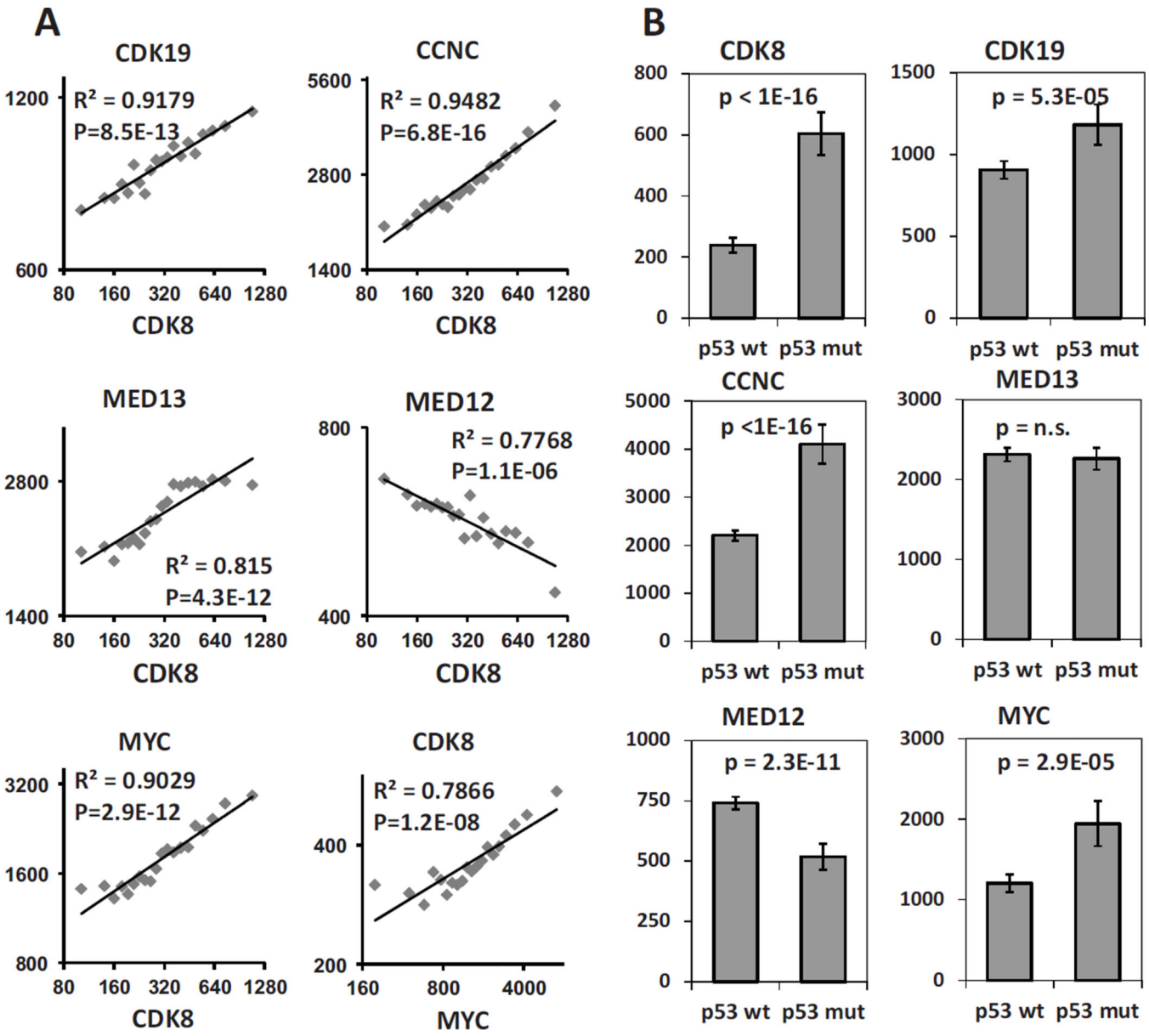

Fig. (4).

Intergenic correlations of the expression of CDK8 and its interactive genes in Affymetrix breast cancer microarray data. (A) Pairwise correlations of expression levels for the indicated genes in 3,491 cancers: median expression plotted for 35 bins of 100 samples assembled into bins according to the levels of the first gene (X-axis); the second gene (median expression in the bin shown on the Y-axis) is labeled at the top of each graph. p-values are based on Spearman rank correlation. (B) Expression levels of the indicated genes in p53 wild-type and p53-mutant tumors in 319 patients with known p53 status. The columns represent the average gene expression; the error bars represent the 95% confidence interval. p-values are based on Mann-Whitney U-test.