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Cell surface GRP78 and Dermcidin cooperate to regulate breast cancer cell migration through Wnt signaling

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Abstract

The heat shock protein GRP78 typically resides in the endoplasmic reticulum in normal tissues, but it has been shown to be expressed on the cell surface of several cancer cells, and some stem cells, where it can act as a signaling molecule by not-yet-fully defined mechanisms. Although cell surface GRP78 (sGRP78) has emerged as an attractive chemotherapeutic target, understanding how sGRP78 is functioning in cancer has been complicated by the fact that sGRP78 can function in a cell-context dependent manner, with a diverse array of reported binding partners, to regulate a variety of cellular responses. We had previously shown that sGRP78 was important in regulating pluripotent stem cell (PSC) functions, and hypothesized that embryonic-like mechanisms of GRP78 were critical to regulating aggressive breast cancer cell functions. Here, using proteomics we identify Dermcidin (DCD) as a novel sGRP78 binding partner common to both PSCs and breast cancer cells. We show that GRP78 and DCD cooperate to regulate stem cell and cancer cell migration that is dependent on the cell surface functions of these proteins. Finally, we identify Wnt/β-catenin signaling, a critical pathway in stem cell and cancer cell biology, as an important downstream intermediate in regulating this migration phenotype.

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DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare no competing interests.

INTRODUCTION

Often occurring years after removal of the primary tumor, cancer recurrence at secondary locations accounts for the majority of all cancer-related deaths [1]. Reports have shown evidence of embryonic stem cell programs in cancer cells that contribute to aggressive malignancy [2-5]. We recently examined pluripotent stem cells (PSCs) in parallel with cancer cells to gain insight into embryonic mechanisms that are exploited in cancer. Specifically, we examined the function of the heat shock protein glucose-regulated protein 78 (GRP78; also known as heat shock 70 kDA protein 5, HSPA5). Although typically localized in the endoplasmic reticulum to assist in protein folding [6, 7], GRP78 has been shown to be localized to the cell surface of cancer cells [7], and as we and others have shown, various types of stem cells [8-11]. While it is established that GRP78 is aberrantly expressed on the cell surface of many cancer cell types, since GRP78 does not contain predicted classical transmembrane domains, it has been reported to exist as a peripheral protein on the cell surface of cancer cells [7, 12]. Evidence supports the concept that sGRP78 relies on interactions with other membrane anchored or transmembrane proteins, as it has been shown to pair with a variety of cell surface binding partners, in a cell context-dependent manner, to mediate varied biological functions [6, 7, 9, 13-15].

We had previously shown that sGRP78 was important in regulating stemness of both pluripotent and breast cancer cells, and proposed that embryonic-like mechanisms of GRP78 were critical to regulate the increased metastatic potential of $sGRP78^{+}$ breast cancer subpopulations we had observed *in vivo* [8]. Thus, we hypothesized that by identifying the sGRP78-mediated mechanisms utilized by cancer that are common to PSCs, that this would reveal the specific sGRP78 mechanisms that lead to the most aggressive cancer outcomes. To accomplish this, we used cell surface proteomics to identify potential common binding partner(s) of sGRP78 between PSCs and breast cancer cells, and have discovered Dermcidin (DCD) as a novel sGRP78 binding partner common to both cell types. The DCD protein is typically expressed in human eccrine sweat glands, where it is secreted as an antimicrobial peptide, and functions as a main defense of the skin against pathogenic organisms [16]. Interestingly, however, DCD is also a presumed oncogene that has been reported to have important functions in cancer [17, 18]. Similar to what has been observed for GRP78, increased expression of DCD has also been linked to advanced cancer stage and an overall poor clinical prognosis in invasive breast carcinomas [17, 19]. Here, we examined the functions of GRP78 and DCD in breast cancer, and show that GRP78/DCD cooperatively regulate breast cancer cell migration, through a Wnt/β-catenin-dependent mechanism.

RESULTS AND DISCUSSION

DCD is a novel cell surface GRP78 binding partner in iPSCs and breast cancer cells

To gain insight into the mechanism by which cell surface GRP78 (sGRP78) is mediating functions in pluripotent and aggressive breast cancer cells that we had previously observed, we used cell impermeable biotin labeling, coupled with immunoprecipitation and mass spectrometry based proteomics to identify potential common binding partners for GRP78 on the cell surface of iPSCs and breast cancer cells (Figure 1A). We identified protein candidates that were common to both iPSCs and breast cancer cells, and after filtering

analysis, only one common sGRP78 binding partner remained, the protein Dermcidin (DCD) (Figure 1A; See Supplementary Materials and Methods).

When examining expression of DCD and GRP78 in both cell types, we found that GRP78 and DCD have similar patterns of expression and distribution. DCD appears to be distributed throughout the cytoplasm of fibroblast cells, but colocalizes at the cell surface with GRP78 in iPSCs (more uniformly) and breast cancer cells (in a subset of cells), in agreement with what we have previously shown for the localization of GRP78 in these cell types (Figure 1B; Supplementary Figures 1 and 2) [8]. Colocalization of GRP78 and DCD at the cell surface of breast cancer cells was further validated through protein analysis in conjunction with a cell surface staining dye and quantified using Manders' Colocalization Coefficient (MCC) analysis [20, 21]. Under basal conditions, the breast cancer cell lines MDA-MB-231 (basal subtype [22]) or MCF7 (luminal A subtype [22]) both show evidence of colocalization of GRP78 and DCD at the cell surface (measured by the cell surface stain MemBrite™ (MB), in blue), as shown by MCC values of ~0.4, with MDA-MB-231 cells displaying slightly higher MCC values than MCF7 cells (Figure 1B, 1C; Supplementary Figure 2). Next, we examined the effects of inducing GRP78, through overexpression of an RFP-GRP78 fusion protein that is under the control of a doxycycline promoter [8]. [Note: this inducible GRP78 system leads to an increase in GRP78 levels that is similar to those caused in physiological conditions, such as glucose starvation and stress; Supplementary Figure 3]. Both breast cancer cells showed an increase in GRP78 and DCD expression, and colocalization at the cell surface, following GRP78 induction (Figure 1B-1D; Supplementary Figure 2). Interestingly, however, when GRP78 levels were induced in MDA-MB-231 cells – which have a very high percentage of $CD24^{-}/CD44^{+}$ breast tumor initiating cell populations (TICs; also commonly referred to as "cancer stem cells") – colocalization of GRP78 and DCD at the cell surface increased substantially (i.e. MCC values went from ~0.4 to ~0.8; Figure 1C), whereas the increase present in MCF7 cells (which display lower percentages of TICs), was more modest (Supplementary Figure 2B) [8]. Total DCD levels also increased after GRP78 induction, as judged by immunofluorescence analysis of protein levels (Supplementary Figure 1C and data not shown). This suggests a possible role for GRP78 in stabilizing and/or regulating the expression of DCD.

We have previously reported that the $CD24^{-}/CD44^{+}$ breast TIC populations express higher levels of GRP78 compared to non-TIC ($CD24^{+}/CD44^{+}$) populations [8]. Additionally, we showed that $sGRP78^{+}$ breast cancer cells are enriched for stemness genes [8]. To determine if DCD was enriched in stem/stem-like populations, we measured DCD transcript levels in isolated TICs, $sGRP78^{+}$ cells, and induced pluripotent stem cells (iPSCs). iPSCs showed a 15-fold increase in DCD transcript levels relative to their fibroblast somatic source (Figure 1E), TICs had a (~5 fold) increase (Figure 1F), and strikingly, $sGRP78^{+}$ cells demonstrated a 15-fold increase (Figure 1G) than their respective controls (Supplementary Table 1). These collective findings reveal that DCD levels are higher in stem/stem-like populations, and demonstrate a correlation between GRP78 and DCD in both expression, and colocalization at the cell surface, in stem cells and breast cancer cells. The expression of DCD in iPSCs and cancer subpopulations with increased stemness is interesting in that DCD has not been shown to be expressed in pluripotent or stem-like contexts before. It supports the hypothesis that aggressive cancer cells could be repurposing a previously unknown embryonic

mechanism role for DCD to promote tumorigenesis. In fact, a previous study suggests that a function of DCD could be to promote a progenitor like phenotype in breast cells, since it was shown that loss of DCD expression in the MDA-MB-361 breast cancer line led the cells to exhibit a more differentiated luminal epithelial phenotype [19].

Cell surface GRP78 and DCD are both important for GRP78-dependent migration

Our previous studies revealed that sGRP78^{+} breast cancer cells demonstrated significantly enhanced metastatic potential *in vivo* [8]. Given the importance of cell migration in cancer metastasis, we next sought to investigate the potential role of GRP78 and DCD in stem cell and breast cancer cell migration. We first performed a transwell assay to investigate if GRP78 and/or DCD were important for regulating iPSC invasion. Inhibition of either cell surface DCD (sDCD) or sGRP78 function, using previously reported inhibitory antibodies [9, 19], each significantly abrogated iPSC migration through the transwell, and at similar levels (Figure 2A). These results show that both sGRP78 and sDCD function are important in regulating PSC invasion.

In breast cancer cells, treatment with an inhibitory GRP78 antibody under basal conditions did not impact cell invasion. [Note: This is likely due to the fact that iPSCs are homogeneous in displaying high sGRP78 expression, whereas breast cancer cells vary in their respective basal sGRP78 levels]. However, inducing GRP78 expression provided a significant invasion advantage, resulting in an ~2-fold increase in the number of breast cancer cells migrating through the transwell (Figure 2B). We and others have shown that inducing GRP78 levels in cancer cells increases levels of GRP78 at the cell surface (Figures 1B, 1C; Supplementary Figures 2 and 3) [6-8, 12]. Therefore, by inducing GRP78 in breast cancer cells, it is likely that this is enabling a more sGRP78-mediated cellular response throughout the cell population, that results in a pronounced cell migration phenotype. This is supported by the fact that inhibiting cell surface GRP78 following GRP78-induction prevented any observed increase in cell migration (see Figure 2D, 2F). Interestingly, inhibiting sDCD function abrogated cell invasion, but only when GRP78 levels were overexpressed, as inhibition of sDCD function in control cells had no effect on cell migration (Figure 2B). These results demonstrate a functional connection between GRP78 and DCD, showing that sDCD function is important in regulating GRP78-dependent invasion.

The transwell assay measures directed cell migration through a porous membrane. To further investigate the importance of GRP78/DCD in regulating migration, we next examined random cell migration, using live-cell time-lapse confocal microscopy experiments. We tracked the migration path of individual cells in MDA-MB-231 cells overexpressing GRP78 in the presence or absence of sDCD inhibition (Figure 2C). Inhibiting sDCD function resulted in a reduced surface area explored by the cancer cells as depicted by illustrating the total path tracks from the origin for the cells in each treatment (Figure 2C). Cell persistence and cell migration efficiency were also reduced when inhibiting sDCD function, as measured by directionality ratio and mean square displacement, respectively (Figure 2C). As

a separate measure of migration, we also utilized the wound healing (i.e. scratch) assay to investigate the regulation of cell migration by GRP78 and DCD. Similar to what we had observed for our invasion assay, overexpression of GRP78 levels provided a significant migration advantage and ability to close the scratch wound at the 72-hour time-point for both MDA-MB-231 (Figure 2D, 2H) and MCF7 (Figure 2F, 2J) breast cancer cells. We show that inhibition of sGRP78 function abrogates cell migration only when GRP78 levels are overexpressed in both MDA-MB-231 and MCF7 breast cancer cells, as inhibition brings the percent gap closure back to baseline levels (Figure 2D-2G). Importantly, inhibition of sDCD function also significantly decreased the gap closure rate of MDA-MB-231 and MCF7 cells back to baseline levels only when GRP78 levels were overexpressed (Figure 2H-2K). These collective findings reveal that cell surface GRP78 and DCD function are both important in regulating GRP78-dependent migration.

Cell surface GRP78/DCD are functionally linked to regulate cancer cell migration

To further decipher the mechanisms of DCD and GRP78-regulated cell migration, we next created a cell line that overexpressed DCD. We transfected MDA-MB-231 cells and MCF7 cells (that contained doxycycline-inducible GRP78) with a plasmid containing DCD-FLAG, and performed drug selection to generate stable lines that overexpressed DCD. Both DCD transcript levels and protein levels were elevated in MDA-MB-231-GRP78^{ind} and MCF7-GRP78^{ind} cells that contained DCD-FLAG (Supplementary Figure 4). As further confirmation, protein expression of FLAG and DCD were also shown to colocalize (Supplementary Figure 4).

In our next group of experiments, we utilized the scratch wound migration assay to examine the effects of overexpressing GRP78, DCD, or both at the same time, on cell migration. We also examined the importance of cell surface functions of GRP78 and DCD proteins in mediating any observed cell migration phenotype changes under these various conditions. MDA-MB-231 cells grown under basal conditions were not affected by treatment with either GRP78 or DCD inhibitory antibody (blue bars in Figure 3A), whereas inducing GRP78 expression significantly increased cell migration that was abrogated by either sGRP78 or sDCD inhibition (green bars in Figure 3A show 72 hour timepoint; Figure 3B shows datapoints across all timepoints), as expected and in agreement with the previous data (Figure 2). Similar to overexpressing GRP78, overexpressing DCD in breast cancer cells led to a significant increase in gap closure rate, illustrating the importance of DCD function in regulating migration (red bars, Figure 3A). Interestingly, the DCD-dependent increase in cell migration was dependent on both sGRP78 and sDCD-function, since inhibition with anti-DCD or anti-GRP78 antibodies brings the gap-closure rate back to the levels seen in untreated cells (red bars, Figure 3A). These results show that cell surface GRP78 and cell surface DCD function are both important in regulating DCD-dependent cell migration. These phenotypes were also true when GRP78 and DCD were both overexpressed (gray bars, Figure 3A). Importantly, however, a synergistic increase in cell migration was not observed, and inhibiting the cell surface function of either protein reduced migration back to baseline levels (gray bars, Figure 3A), suggesting that both proteins are jointly required for a migration response.

In agreement, the increase in cell migration after GRP78 or DCD induction in MCF7 cells was also reduced back down to baseline levels with sDCD inhibition (Supplementary Figure 5A). Furthermore, when using a separate retroviral system to overexpress GRP78 that did not rely on doxycycline treatment (Supplementary Figures 5B-F), the same phenotypes were observed. Specifically, MDA-MB-231 cells overexpressing GRP78 showed an increase in cell migration, that was not present in a retroviral-matched GFP expressing control cell line (Supplementary Figure 5F), Moreover, when GRP78 or DCD was overexpressed, sDCD inhibition abrogated the observed cell migration (Supplementary Figure 5F). A parallel phenotype was observed when overexpressing GRP78 or DCD in an additional breast cancer cell line, the HER2+ cell line SK-BR-3 [22] (Supplementary Figure 5F). Collectively, these data show that cell surface GRP78 and DCD levels are important in regulating both GRP78-dependent and DCD-dependent cell migration, and support a novel functional link/interaction between these two cell surface proteins in regulating breast cancer cell migration.

GRP78/DCD regulate breast cancer cell migration through Wnt/β-catenin signaling

We next investigated the downstream mechanisms that GRP78/DCD could be utilizing to regulate breast cancer cell migration. We had previously shown that sGRP78^{+} breast cancer cells were enriched for genes involved in Wnt signaling [8]. The canonical Wnt signaling pathway plays critical roles in various aspects of stem cell and cancer cell function, including cell migration [23, 24]. Therefore, we decided to investigate the importance of the Wnt pathway in the regulation of cell migration through surface GRP78/DCD.

We first examined active β-catenin protein levels, and found that they were increased ~7-fold in DCD-overexpressing MDA-MB-231 cells, which was further enhanced to ~30-fold following Wnt signaling activation with CHIR99021 (a GSK-3 inhibitor) treatment (Figure 4A). Breast cancer cells with increased levels of DCD were also enriched for transcript expression of Wnt target genes/Wnt signaling pathway genes (Figure 4B, see Supplementary Materials and Methods; Supplementary Figure 6; Supplementary Table 1). To measure if these changes reflected an increase in β-catenin transcriptional activity in control and DCD-overexpressing cells, MDA-MB-231 cells were transfected with plasmids containing Luciferase under the control of normal TCF/LEF promoter sequence (TOPFlash) or mutated TCF/LEF sequence (FOPFlash) to serve as a negative control, and Renilla luciferase plasmids as a transfection control. Thus, in this assay, the ratio of TOPFlash luciferase activity to Renilla Luciferase activity can be used as a measure of β-catenin transcriptional activity [25].

β-catenin transcriptional activity was significantly increased following DCD-overexpression, and was further enhanced by Wnt signaling activation by CHIR99021 treatment (Figure 4C). Interestingly, the increase in β-catenin transcriptional activity induced by CHIR99021, DCD-FLAG, or DCD-FLAG + CHIR99021 combined, were all dependent on surface GRP78 and DCD function, as treatment with inhibitory antibodies abrogated the increase in β-catenin transcriptional activity in each condition where it was elevated (Figure 4D). These data suggest that Wnt/β-catenin signaling and transcriptional activity is downstream of surface GRP78/DCD function. We further show that the GRP78-dependent migration, DCD-dependent migration, and combined GRP78/DCD-dependent migration (as measured by

wound healing migration assays), is dependent on β-catenin activity, since treatment with the chemical XAV939 β-catenin/Wnt signaling inhibitor abrogated the increase in gap closure rate induced by these conditions (Figure 4E; Supplementary Figure 6). These collective results suggest that sGRP78 and DCD cooperate to regulate breast cancer cell migration through β-catenin dependent-Wnt signaling (Figure 4F). This novel function for GRP78/DCD as regulators of the Wnt/β-catenin pathway is very intriguing considering the importance of the Wnt pathway in regulating critical components of stem cells/pluripotency, development and cancer. Future work will focus on further dissecting the specific mechanisms of regulation of the Wnt pathway by GRP78/DCD, in both PSCs and cancer.

MATERIALS AND METHODS

Cell Lines and Reagents

MCF7, MDA-MB-231, SK-BR-3 and IMR90 cells were purchased from American Type Culture Collection (ATCC). MDA-MB-231-GRP78^{ind} cells [8] (Supplementary Figure 7), and human iPSC lines [26, 27] were generated as previously reported. All cells were cultured as described previously [8]. DCD-FLAG construct (ORF clone OHu30172) was purchased from Genscript. M50 Super 8x TOPFlash and M51 Super 8x FOPFlash was a gift from Randall Moon (Addgene plasmid #12456). pHRL-SV40 was obtained from Promega. CHIR99021 was purchased from StemCell Technologies (#72054). XAV939 and ROCK inhibitor Y-27632 were purchased from Sigma (#X3004 and #SCM075). GRP78-N20 (#sc-1050) and DCD-N20 (#sc-27465) antibodies, or Goat-IgG controls (#sc-2028), used in inhibitory experiments, were purchased from Santa Cruz Biotechnology.

Remaining Materials and Methods are available as a Supplementary File.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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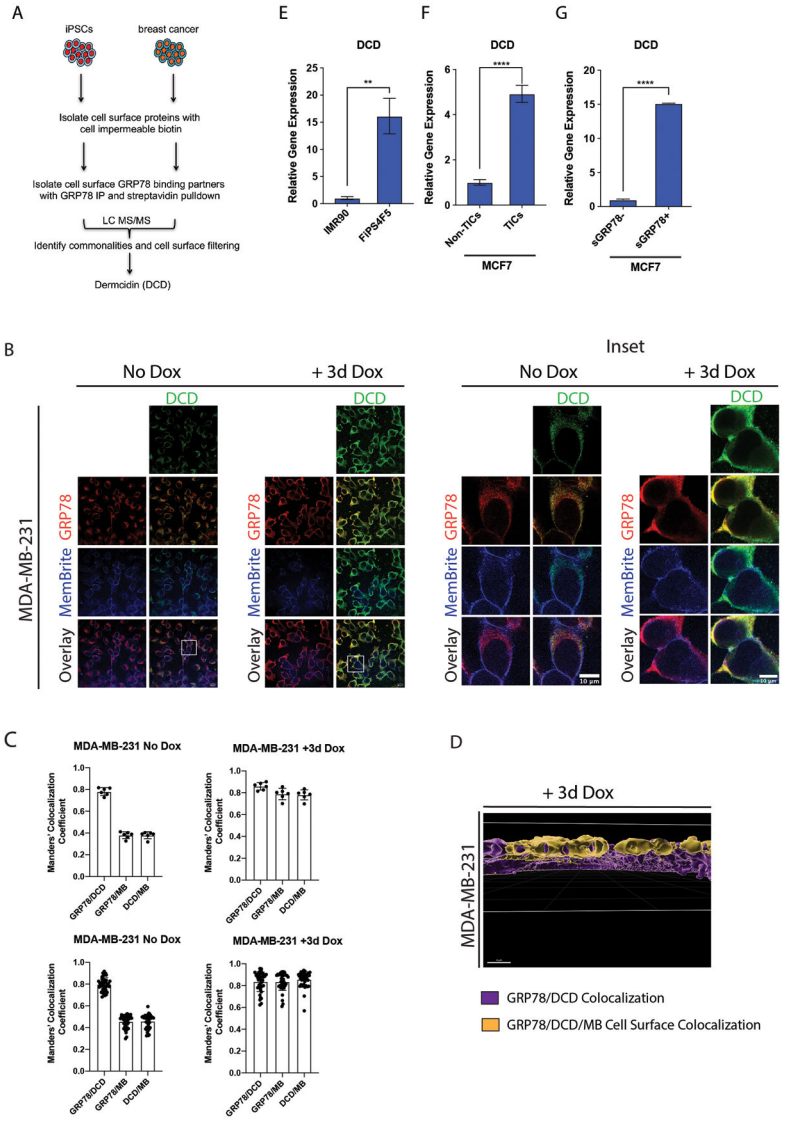

Figure 1. DCD and GRP78 colocalize on the surface of iPSCs and breast cancer cells.

(A) Dermcidin was an identified potential binding partner from a mass spectrometry proteomic screen profiling common surface GRP78 binding partners between iPSCs and cancer cells. (B) MDA-MB-231-GRP78^{ind} cells were grown in the absence or presence of Doxycycline for 3 days, then fixed and stained with MemBrite™ (blue) (MB), and antibodies to DCD (green) or GRP78 (red) to examine colocalization on the surface of breast cancer cells using confocal microscopy. Images were taken at 60x magnification. Insets of images are shown to the right of each panel. (C) To assess colocalization of GRP78 and DCD at the cell surface of MDA-MB-231-GRP78^{ind} cells under basal conditions and following GRP78 overexpression, Manders' Colocalization Coefficient (MCC) values were calculated for GRP78/DCD, GRP78/MB, and DCD/MB colocalization. Bar charts represent results from three independent experiments. Graphs depict whole fields of view (top; each value represents 1 field; results from 6 fields shown) or from individual cells from the field images (bottom; each value represents 1 cell; 40 cells shown, obtained from the 6 fields).

(D) MDA-MB-231-GRP78^{ind} cells that had been treated with doxycycline for 3 days and stained with MemBrite™ (MB), anti-DCD or anti-GRP78 antibodies as described were used in confocal microscopy. Z-stack images were taken using a 100x objective and 2.5x optical zoom. 3-D rendering analysis of the X-Z plane to visualize cell surface colocalization was performed using Imaris Software. Examples of colocalization between DCD and GRP78 (purple), and cell surface colocalization of DCD and GRP78 (GRP78/DCD/MB; gold) are shown. (E-G) Relative DCD gene expression was examined between (E) IMR90 and FiPS4F5, (F) MCF7 Non-TICs ($CD24^+/CD44^+$) and TICs ($CD24^-/CD44^+$), as well as (G) MCF7 $sGRP78^-$ and $sGRP78^+$ cell populations using real-time PCR. Statistical significance was calculated using a student's t-test. ** = $p<0.01$, **** = $p<0.0001$.

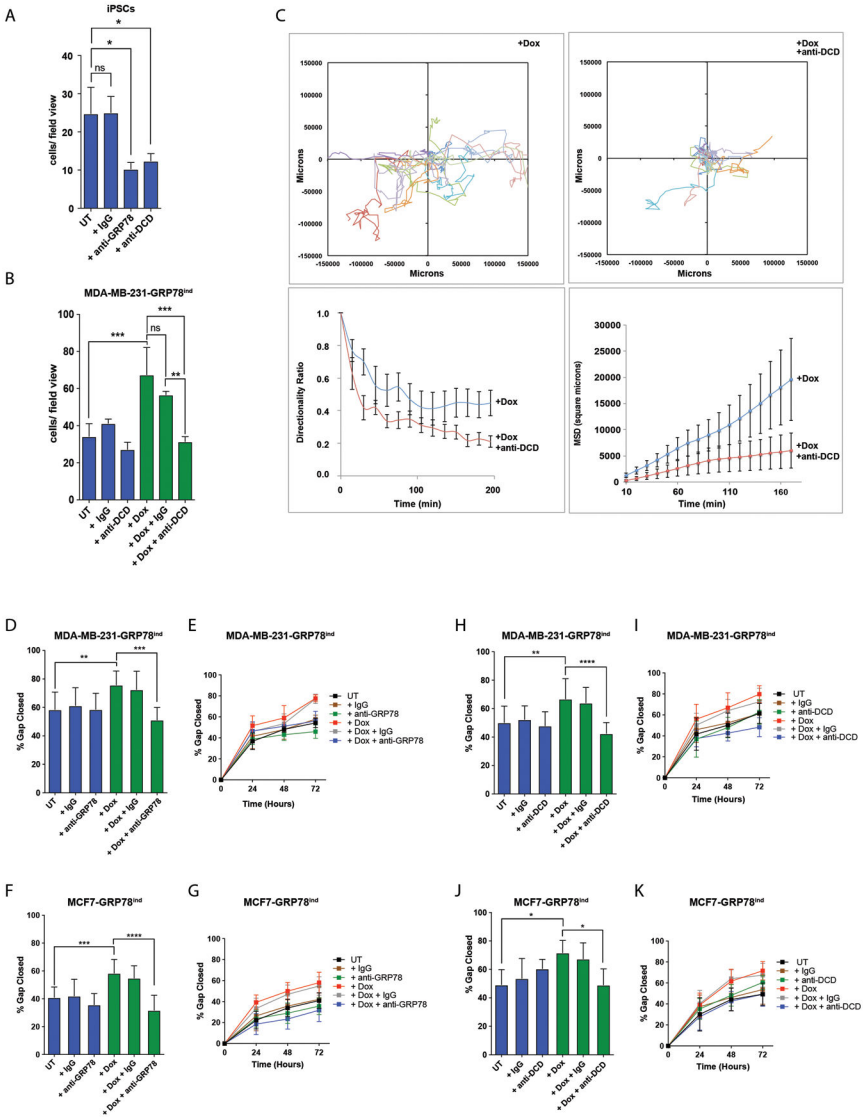

Figure 2. Cell surface GRP78 and DCD are both important for GRP78-dependent migration.

(A) HUViPS4F1 cells were seeded into the upper compartment of a Transwell chamber and treated with goat IgG, anti-GRP78 or anti-DCD (1μg/mL). Lower compartments were supplemented with media and inserts were incubated for 24h. Inserts were fixed, cells in the upper chamber were removed and remaining migrating cells were fluorescently stained and counted. Bars represent mean ± SD (n=3). Statistical significance was calculated using one-way ANOVA. * = $p<0.05$ (B) MDA-MB-231-GRP78^{ind} cells +/− doxycycline were seeded into the upper compartment of a Transwell chamber in serum free DMEM media only (untreated, UT), or serum free DMEM media treated with goat IgG, or anti-DCD (1μg/mL). Lower compartments were supplemented with DMEM media + 20% FBS and inserts were incubated for 24 hours. Inserts were fixed, cells in the upper chamber were removed, and remaining migrating cells were fluorescently counted. Bars represent mean ± SD (n=3). Statistical significance was calculated using one-way ANOVA. ** = $p<0.01$; *** = $p<0.001$ (C) MDA-MB-231-GRP78^{ind} cells were pre-treated for three days with doxycycline. Cells

were imaged for RFP expression, in the absence or presence of anti-DCD (1μg/mL), every 10 minutes for 4.5 hours using live cell time-lapse confocal microscopy. Random cells were manually tracked and cell trajectories from the origin, and mean square displacement and directionality ratio were analyzed. (D-K) MDA-MB-231-GRP78^{ind} or MCF7-GRP78^{ind} cells were pre-treated with doxycycline for 24 hours and then seeded to confluency. A vertical and horizontal wound was generated in each well. Cells were then grown in serum free media only +/− doxycycline or treated with goat IgG, anti-GRP78 (D-G), or anti-DCD (H-K) (1μg/mL). Images were taken every 24 hours for 72 hours. The percentage of wound closure at 72 hours is shown in D, F, H, and J. Bars represent mean ± SD (n=3). The percentage of wound closure for all time points is shown in E, G, I, and K. Line graphs represent wound closure rates at all experimental time points. Statistical analysis was performed using one-way ANOVA, * = $p<0.05$; ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

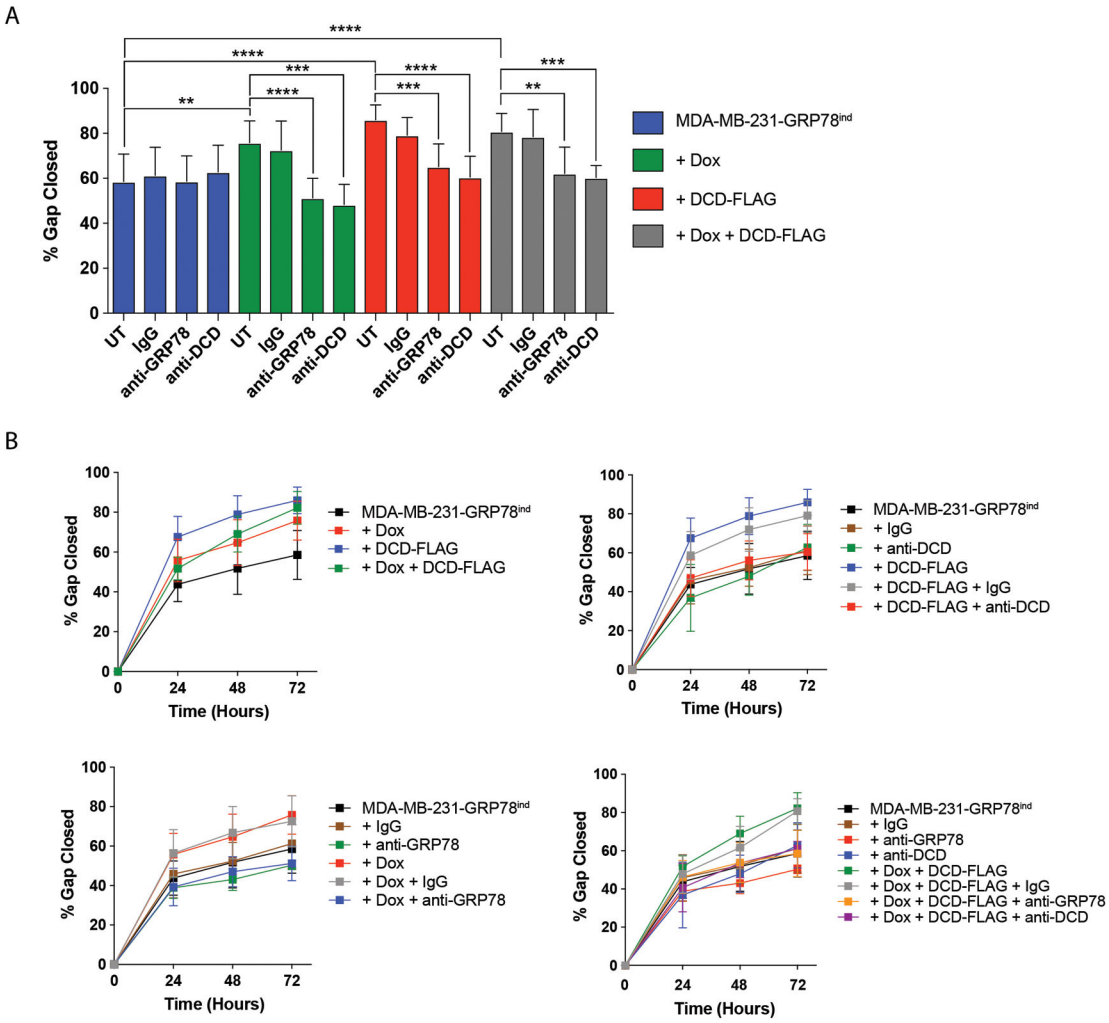

Figure 3. Cell surface GRP78 and DCD are functionally linked to regulate cancer cell migration.

(A) MDA-MB-231-GRP78^{ind} or MDA-MB-231-GRP78^{ind} + DCD-FLAG cells were pre-treated with doxycycline for 24 hours and then seeded to confluency. A vertical and horizontal wound was generated and cells were then grown in serum free media only +/− doxycycline or treated daily with goat IgG, anti-GRP78, or anti-DCD (1μg/mL). The percentage of wound closure at 72 hours is shown in A. Bars represent mean ± SD (n=3). (B) Line graphs displaying the percentage of wound closure across all experimental time points. Statistical analysis was performed using one-way ANOVA. ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

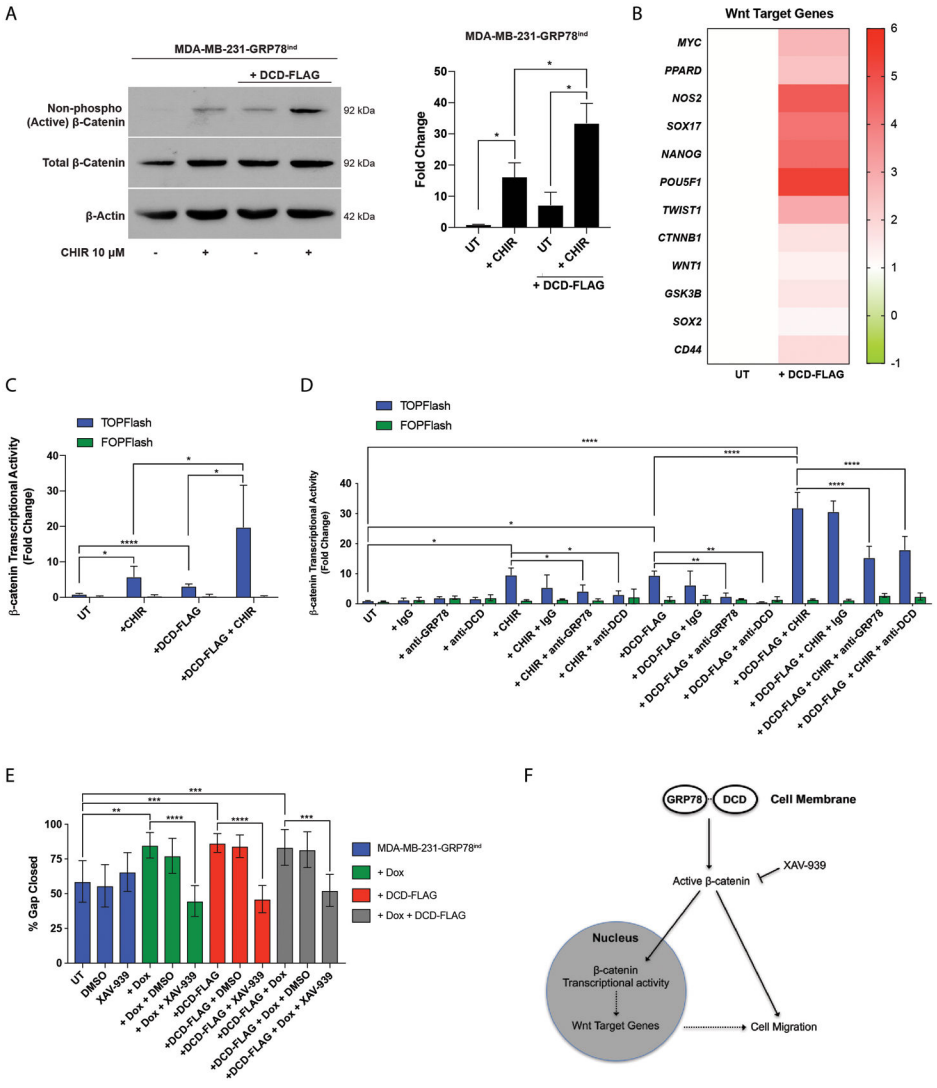

Figure 4. GRP78 and DCD regulate breast cancer cell migration through Wnt/β-catenin signaling.

(A) MDA-MB-231-GRP78^{ind} and MDA-MB-231-GRP78^{ind} + DCD-FLAG cells were serum deprived for 24 hours (untreated, UT), or treated with 10μM CHIR99021 for 30 minutes, and then lysed. Cell lysates were separated using SDS-PAGE and probed for active β-catenin, total β-catenin, and β-actin protein levels using immunoblot analysis. Quantification of active β-catenin levels compared to β-actin levels is shown on the right. Statistical significance was calculated using a student's t-test. * = $p<0.05$. (B) Relative amounts of Wnt target genes were analyzed between MDA-MB-231-GRP78^{ind} and MDA-MB-231-GRP78^{ind} + DCD-FLAG cells using real-time PCR. (C) MDA-MB-231-GRP78^{ind} and MDA-MB-231-GRP78^{ind} + DCD-FLAG cells were seeded in a 96-well plate and transfected with TOPFlash/FOPFlash plasmids. β-catenin transcriptional activity was assessed after 24-hour treatment with 10μM CHIR99021 by measuring luminescence. TOPFlash values are presented as fold change from untreated cells. Bars represent mean ± SD (n=3). * Indicates significance, $p<0.05$, **** = $p<0.0001$ analyzed with Welch's ANOVA. (D) MDA-MB-231-GRP78^{ind} and MDA-MB-231-GRP78^{ind} + DCD-FLAG cells

were seeded in a 96-well plate and transfected with TOPFlash/FOPFlash plasmids. Cells were pretreated for 3 hours with IgG control, anti-GRP78, or anti-DCD inhibitory antibodies (1μg/mL). β-catenin transcriptional activity was assessed after 24-hour treatment with 10μM CHIR99021 by measuring luminescence. TOPFlash values are presented as fold change from untreated cells. Bars represent mean ± SD. * indicates significance, $p<0.05$, ** = $p<0.01$, **** = $p<0.0001$ analyzed using an unpaired two-tailed t-test. (E) MDA-MB-231-GRP78^{ind} or MDA-MB-231-GRP78^{ind} + DCDFLAG cells were pre-treated with doxycycline for 24 hours and then grown to confluency. A vertical and horizontal wound was generated and cells were then grown in serum free DMEM media only +/− doxycycline or treated daily with DMSO, or 10μM XAV-939. The percentage of wound closing compared to the initial wound was calculated for each time point. Displayed is the percent gap closure rate at 72 hours. Bars represent mean ± SD (n=3). ** indicates significance compared to untreated cells, $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$ using a Kruskal-Wallis test. (F) Schematic of proposed model. GRP78 and DCD interact at the cell surface – either directly, or indirectly through unknown cofactor(s) – to regulate cell migration by activating the canonical Wnt signaling pathway.