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Junctional Adhesion Molecule A is co-expressed with HER2 in breast tumors and acts as a novel regulator of HER2 protein degradation and signaling

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Running Title: JAM-A regulates HER2 protein degradation

Abbreviations:

DMSO, Dimethylsulphoxide; FBS, Foetal Bovine Serum; HER2, Human Epidermal growth factor Receptor 2; JAM-A, Junctional Adhesion Molecule–A; MEM, Minimum Essential Medium; SDS-PAGE, Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis; TMA, Tissue Microarray.

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Abstract

Junctional Adhesion Molecule-A (JAM-A) is a membranous cell-cell adhesion protein involved in tight junction formation in epithelial and endothelial cells. Its over-expression in breast tumors has recently been linked with increased risk of metastasis. We sought to identify if JAM-A over-expression was associated with specific subtypes of breast cancer as defined by expression of human epidermal growth factor receptor-2 (HER2), estrogen receptor (ER) and progesterone receptor (PR). To this end, JAM-A immunohistochemistry was performed in two breast cancer tissue microarrays. In parallel, cross-talk between JAM-A, HER2 and ER was examined in several breast cell lines using complementary genetic and pharmacological approaches. High JAM-A expression correlated significantly with HER2 protein expression, ER-negativity, lower patient age, high grade breast cancers and aggressive luminal B, HER2 and basal subtypes of breast cancer. JAM-A and HER2 were co-expressed at high levels *in vitro* in SKBR3, UACC-812, UACC-893 and MCF7-HER2 cells. Knockdown or functional antagonism of HER2 did not alter JAM-A expression in any cell line tested. Interestingly however, JAM-A knockdown decreased HER2 and ER-α expression, resulting in reduced levels of phospho- (active) AKT without effect on ERK phosphorylation. The downstream effects of JAM-A knockdown were reversed upon treatment with the proteasomal inhibitor MG132. We conclude that JAM-A is co-expressed with HER2 and associates with aggressive breast cancer phenotypes. Furthermore we present novel evidence that JAM-A regulates HER2 proteasomal degradation and activity, suggesting that JAM-A may offer promise as a therapeutic target in HER2-positive breast cancers.

Key words: junctional adhesion molecule-A; breast cancer; tight junction; human epidermal growth factor receptor 2, AKT, estrogen receptor

Introduction

Breast cancer is the most common form of cancer among women in North America and Europe. Each year it is diagnosed in over 1 million women worldwide, and is the cause of death of over 450,000 (Ferlay J, 2010). A major challenge in breast cancer treatment is to identify those patients most likely to develop recurrence so that appropriate therapy can be selected. Genomic studies have established five major breast cancer intrinsic subtypes (luminal A, luminal B, HER2-enriched, basal-like and claudin-low) and a normal breast-like group that show significant differences in incidence, survival and response to therapy (Carey *et al.*, 2006; Herschkowitz *et al.*, 2007; Perou *et al.*, 2000; Sorlie *et al.*, 2001).

Estrogen receptor (ER)-negative (HER2-enriched, basal-like and claudin-low) subtypes are associated with an increased risk of aggressive metastatic disease when compared to luminal (ER-positive) subtypes. HER2 is amplified in 20–30% of invasive breast cancers, which represent the luminal B and HER2-enriched subtypes (Slamon *et al.*, 1987). HER2 oncogenic properties are thought to result from increased activation of several signaling pathways including the extracellular signal-related kinase (ERK) cascade and the phosphoinositide 3-kinase (PI3K)-AKT pathway (Baselga and Swain, 2009). Therapeutic inhibition of HER2 function has been successfully applied to treat patients with HER2-positive breast cancer (Geyer *et al.*, 2006; Piccart-Gebhart *et al.*, 2005; Romond *et al.*, 2005); however the efficacy of such strategies is limited by primary and acquired resistance (Baselga and Swain, 2009).

Growth factor receptors are not the only membrane proteins of interest in the pathophysiology of breast cancer. Tight junction (TJ) proteins such as JAM-A, CAR, claudin -3 and -4 have been shown to be over-expressed in breast cancer (Hewitt *et al.*, 2006; Hoevel *et al.*, 2004; Lanigan *et al.*, 2009; Martin *et al.*, 2005; McSherry *et al.*, 2009; Osanai *et al.*, 2007a; Osanai

et al., 2007b), and may represent novel therapeutic targets. In normal breast tissue these proteins are expressed at lower levels and confined predominately to areas of epithelial cell-cell contact. Enhanced expression in tumor cells may make TJ proteins interesting candidates for targeted delivery of cytotoxic agents, or for downregulation of signaling pathways related to oncogenic functions.

TJs along with adherens junctions form the apical junctional complex, which encircles the apex of epithelial and endothelial cells and acts to maintain cell polarity by separating apical and basolateral compartments (Nelson, 2003). Of primary interest in our study is the TJ protein JAM-A, which has important functions in cellular adhesive processes including TJ assembly (Liang *et al.*, 2000), cellular polarity (Ebnet *et al.*, 2001), the regulation of cell morphology (Mandell *et al.*, 2005), angiogenesis (Cooke *et al.*, 2006), platelet activation (Babinska *et al.*, 2002) and leukocyte migration (Ostermann *et al.*, 2002). We (McSherry *et al.*, 2009) and others (Murakami *et al.*, 2011) recently identified a significant association between JAM-A over-expression and poor prognosis of breast cancer patients. Furthermore JAM-A has been demonstrated to be a target of the microRNA miR-145, which is downregulated in breast cancer and whose over-expression decreases motility and invasive behavior in breast cancer cells (Gotte *et al.*, 2010).

In this study we therefore sought to refine the link between JAM-A and aggressive tumor behavior using breast cancer clinical datasets in conjunction with *in vitro* cell line models. We report that high JAM-A expression correlates significantly with HER2 protein expression, ER-negativity and high grade breast cancers. In breast cell lines co-expressing JAM-A and HER2, we present novel evidence that HER2 knockdown or pharmacological antagonism does not influence JAM-A protein expression, whereas JAM-A knockdown reduces that of

HER2 and ER-α via a pathway involving the proteasome. JAM-A knockdown also exerts a selective effect on signaling through HER2 effector pathways, demonstrated as reductions in AKT but not ERK phosphorylation. Collectively, our results suggest that JAM-A is a novel upstream regulator of HER2 degradation and activity, and that its overexpression may promote breast cancer progression in HER2-positive tumors. We submit that JAM-A offers promise not just as a novel biomarker of the HER2-positive breast cancer phenotype, but also as a drug target of the future.

Results and discussion

The intercellular adhesion protein JAM-A is expressed as a homodimer on epithelial and endothelial cell surfaces and has been shown to have important physiological functions in numerous cellular adhesive processes. We and others have recently linked high breast epithelial expression of JAM-A with aggressive disease and poor outcome in breast cancer patients (Gotte *et al.*, 2010; McSherry *et al.*, 2011; McSherry *et al.*, 2009; Murakami *et al.*, 2011). We independently verified these findings on a small breast cancer TMA of 48 tumor cores, in which tissue cores were scored 0, 1+, 2+ or 3+ according to the level of membranous JAM-A staining in tumor cells (**Supplemental Fig. S1**). JAM-A expression was classified as low [0 or 1+] in 28 tumors (58.3%) and high [2+ or 3+] in 20 tumors (41.7%). High JAM-A expression was significantly associated with low progesterone receptor (PR) expression ($p<0.05$), invasive breast cancers ($p<0.05$), HER2+/ER-/PR- expressing breast cancers ($p<0.05$) and breast tumors of ≥2cm in diameter ($p<0.05$; **Supplemental Table 1**).

To further interrogate the sub-types of aggressive tumors associated with high JAM-A expression, we analyzed a larger TMA of 167 tumor cores with known HER2 expression status. Tumor membranous expression of JAM-A was classified as low [0 or 1+] in 75 tumors (44.9%) and high [2+ or 3+] in 92 tumors (55.1%). Examination of the relationship between JAM-A expression and other clinicopathological variables (**Table 1**) as examined by χ^2 test and Fisher's exact tests on SPSS version 15.0 revealed that high JAM-A expression was significantly associated with HER2 protein expression ($p<0.05$), high tumor grade ($p<0.001$) and ER-negativity ($p<0.001$). According to one molecular classification of breast cancers (Sorlie *et al.*, 2001), high JAM-A levels were also associated with the HER2+ and basal subtypes of breast cancer ($p<0.05$). These data indicate that high JAM-A expression in breast

cancer is associated with aggressive breast tumor characteristics, and highlights a role for JAM-A as a possible biomarker.

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Since breast cancer is a heterogeneous disease, we screened JAM-A protein levels in a range of cell lines expressing different profiles of the clinicopathologically-relevant molecular markers HER2 and ER-α (**Supplemental Fig. S2**). All the HER2-positive cell lines expressed moderate to high levels of JAM-A, while the basal-like ER-/HER2-negative cell line MDA-MB-231 expressed low levels of JAM-A. As JAM-A expression was high in several HER2-positive breast cancer cell lines, we questioned whether expression levels of one protein could regulate those of the other. We first knocked down HER2 expression in several breast cancer cell lines using an siRNA approach. As shown by densitometric quantitation in **Fig. 1,** HER2 was successfully knocked down in SKBR3, UACC-893, MCF7-HER2 and LCC1 cells. A representative Western blot for UACC-893 cells is shown. HER2 knockdown had no effect on JAM-A protein expression in the ER-negative cell lines SKBR3 and UACC-893. Interestingly, however, there was a partial reduction in JAM-A protein expression following HER2 knockdown in the ER-positive cell lines MCF7-HER2 (Pegram *et al.*, 1997) and LCC1; **Fig. 1A**).

Subtle reductions in JAM-A expression secondary to HER2 knockdown in ER-positive cells suggested that JAM-A expression levels may be regulated by those of ER-α. We thus knocked down ER-α in conjunction with HER2 in MCF7-HER2 and LCC1 cells to examine if JAM-A expression could be restored. As shown in the densitometric quantitation in **Fig. 1B,** ER-α knockdown either alone or in conjunction with HER2 knockdown did not alter JAM-A protein expression in either cell line. A representative blot from LCC1 cells is shown. Furthermore chemical inhibition of HER2 with AG825 (**Fig. 1C)**, inhibition of ER-α with

tamoxifen (OHT) or stimulation with 17-β-estadiol (E_2; **Fig. 1D)** did not significantly alter JAM-A expression in MCF7-HER2 cells.

Having observed no regulation of JAM-A protein expression following manipulation of HER2 or ER-α, we next tested the opposite strategy. JAM-A was transiently knocked down by siRNA in SKBR3, UACC-893, MCF7-HER2 and LCC1 cells. As shown by densitometric quantitation of western blots **(Fig 2A** graph), JAM-A protein expression was successfully reduced in all cell lines after 72h. HER2 protein expression was partially but not significantly reduced by knockdown of JAM-A in ER-negative SKBR3 and UACC-893 cells. However HER2 and ER-α protein expression were both significantly reduced following knockdown of JAM-A in ER-positive MCF7-HER2 and LCC1 cells **(Fig 2A** graph). A representative blot from LCC-1 cells is shown. We also tested whether functional antagonism of JAM-A using the dimerisation-blocking antibody J10.4 could influence HER2 protein expression in SKBR3 or UACC-893 cells. As shown in **Fig. 2B** (graph)**,** J10.4 had no effect on the expression levels of JAM-A, HER2 or Y1221-phosphorylated HER2 in either cell line. A representative blot from UACC-893 cells is shown.

Following our observation that HER2 and ER-α protein expression was reduced upon knockdown of JAM-A, we next examined whether JAM-A knockdown caused increased turnover of ER-α, HER2 and its effectors AKT and ERK. JAM-A was transiently knocked down by siRNA in MCF7-HER2 cells, whereupon we noted not only a significant reduction in HER2 and ER-α expression but also that of phospho-AKT (DMSO condition in **Fig. 3A,** quantitation in **Fig. 3B**). In contrast there was a variable increase in ERK phosphorylation in response to JAM-A knockdown, but this was not statistically significant. To test whether reductions in HER2 and pAKT expression downstream of JAM-A knockdown reflected

proteasomal degradation, cells were treated with the proteasomal inhibitor MG132 4 hours prior to lysis. The protein expression of HER2, ER-α and phospho-AKT was restored by MG132 treatment of JAM-A knockdown cells (**Fig. 3C**). However phospho-ERK levels was not restored under the same conditions. This suggested that the mechanism whereby JAM-A expression regulates that of HER2 and ER-α is through alteration of proteasomal degradation. This adds to increasing evidence that JAM-A regulates protein turnover through proteasomal degradation, as JAM-A knockdown has previously been shown to similarly reduce the protein expression of β1-integrin (McSherry *et al.*, 2011; McSherry *et al.*, 2009; Severson *et al.*, 2009) and αV- and α5-integrin (McSherry *et al.*, 2011). Further studies are required to determine the specific proteasomal components implicated, but it is intriguing to speculate that JAM-A may also have the capability to likewise regulate the expression of other growth factor receptors/adhesion molecules relevant to breast cancer progression.

Interestingly, the HER2 effectors AKT and ERK were differentially regulated downstream of JAM-A manipulation in our models. Specifically, AKT phosphorylation was significantly reduced upon JAM-A knockdown and restored following proteasomal inhibition, whereas ERK phosphorylation was not. A similar regulation of HER2 signaling has been reported following adenine nucleotide translocase 2 (ANT2) knockdown, which increases HER2 degradation through suppression of HSP90 functions, and results in inhibition of the PI3K/AKT signaling pathway (Jang *et al.*). Active AKT has also been shown to regulate ERK activation by phosphorylating and inhibiting Raf-1, a kinase which activates ERK through MEK (Zimmermann and Moelling, 1999). Our results suggest that JAM-A knockdown regulates AKT indirectly by reducing HER2 protein expression. AKT has been shown to promote cancer cell invasion, cell survival, increased motility and metalloproteinase production as well as promoting resistance to trastuzumab and tamoxifen in breast cancer

cells (Brunet *et al.*, 1999; Clark *et al.*, 2002; Kim *et al.*, 2001). Thus one might predict that the over-expression of JAM-A in aggressive breast cancers (McSherry *et al.*, 2009) is associated with increased levels of AKT activity which promote survival and motility. Furthermore the pharmacological reduction of JAM-A signaling may have benefit in reducing metastatic risk, since we (McSherry *et al.*, 2011; McSherry *et al.*, 2009) and others (Gotte *et al.*, 2010) have shown that JAM-A knockdown or functional antagonism decreases breast cancer cell migration, an early event in metastasis.

It is interesting to note that ER-α expression was regulated by JAM-A in the ER-positive cell lines tested, whereas our TMA data demonstrated a link between high JAM-A expression and ER-α negativity. Based on TMA data we had hypothesized that ER would negatively regulate JAM-A expression, yet pharmacological interference with ER-α signaling using estrogen or tamoxifen did not regulate JAM-A expression in the cell lines tested. This intriguing difference illustrates the importance of considering cell line data in conjunction with more pathophysiologically-representative tissue data from a large number of patients. However since stimulation of ER-β with estrogen has been shown to upregulate JAM-A in intestinal cells (Braniste *et al.*, 2009), we speculate that a level of unexplored crosstalk exists. Given the functionally opposing roles of ER-α and –β in breast tissue, it is possible that ER-α negative tissue may express ER-β, however a larger ER-negative cohort of tissue samples would be needed to answer this question.

In conclusion, we have shown that JAM-A is upregulated in HER2-positive, ER-negative breast cancers and associated with more aggressive breast cancers. While high JAM-A expression in tissue microarrays correlates with ER negativity, we show for the first time that JAM-A regulates HER2 and ER-α proteasomal degradation in breast cell lines. Our data also

indicate that observed reductions in HER2 downstream of JAM-A prevent downstream activation of AKT. This effect on AKT following JAM-A knockdown suggests that therapeutics targeting JAM-A expression or signaling may offer new treatment options for aggressive tumors over-expressing JAM-A and/or HER2.

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Conflict of interest

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1: HER2 or ERα knockdown do not regulate JAM-A expression

(A) Graph: Densitometric quantitation of HER2 and JAM-A protein expression in UACC-893, MCF7-HER2 and LCC1 breast cells transfected for 72h with 25nM of control siRNA (siGENOME non-targeting siRNA #1, Dharmacon/Thermo Scientific, Epsom, UK) or HER2 siRNA (Dharmacon) using the Dharmafect-1 siRNA transfection system (Dharmacon). Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A (Zymed Laboratories Inc., Invitrogen, CA, USA), HER2 (Cell Signaling Technologies, MA, USA) or actin (Abcam, Cambridge, UK) antibodies. A representative Western blot from UACC-893 cells is shown. **(B)** Graph: Densitometric quantitation of JAM-A, ER-α and HER2 protein expression in ER-positive MCF7-HER2 and LCC1 cells transfected for 72h with 25nM of control siRNA (siGENOME non-targeting siRNA #1, Dharmacon), ER-α siRNA (Ambion, Life Technologies, USA) or ER-α + HER2 siRNA using the Dharmafect-1 siRNA transfection system. Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A, HER2, ERα (Santa Cruz, CA, USA) or actin antibodies. A representative Western blot from LCC1 cells is shown. **(C)** Western blot analysis of JAM-A, ER-α and HER2 protein expression in MCF7-HER2 cells treated for 6 hours with vehicle or the HER2 inhibitor AG825 (100μM; Sigma-Aldrich, Poole, UK). Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human HER2-p-1221/1222 (Cell Signaling Technologies, MA, USA), HER2, p-ERK (Cell Signaling Technologies, MA, USA), ERK (Cell Signaling Technologies, MA, USA), JAM-A or actin antibodies. A representative Western blot from MCF7-HER2 cells is shown. **(D)** Western blot analysis of JAM-A expression in MCF7 cells treated for 24 hours with vehicle, 17β-estradiol (E_2, 10nM; Sigma-Aldrich, Poole, UK), tamoxifen (100nM Sigma-Aldrich, Poole, UK) or a combination of both, following preincubation in phenol red-free media containing 10% charcoal dextran-stripped

FBS. Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A and actin antibodies. A representative Western blot from MCF7 cells is shown. In all graphs, error bars refer to standard error of the mean of triplicate experiments.

Figure 2: JAM-A knockdown but not antagonism reduces HER2 and ERα protein expression

(A) Graph: Densitometric quantitation of JAM-A, ERα and HER2 protein expression in SKBR3, UACC-893 cells transfected for 72h with 75nM of control siRNA (MISSION® siRNA Universal Negative control #1, Sigma-Aldrich, Poole, UK) or JAM-A siRNA (SASI_Hs01_00049785, Sigma-Aldrich, Poole, UK) using the N-TER nanoparticle siRNA transfection system (Sigma-Aldrich, Poole, UK). MCF7-HER2 and LCC1 cells transfected for 72h with 25nM of control siRNA (siGENOME non-targeting siRNA #1, Dharmacon) or JAM-A siRNA (SASI_Hs01_00049785, Sigma-Aldrich, Poole, UK) using the Dharmafect-1 siRNA transfection system. Cell extracts were run on SDS–PAGE and immunoblotted with anti-human JAM-A, HER2, ERα and actin antibodies. A representative Western blot from LCC1 cells is shown. **(B)** Graph: Densitometric quantitation of JAM-A, pHER2 (Y-1221) and HER2 protein expression in SKBR3, UACC-893 cells treated for 72h with 10μg/ml JAM-A inhibitory antibody J10.4 or control mouse IgG (sodium azide- and gelatin-free; Santa Cruz, CA, USA). Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A, HER2-p-1221/1222, HER2 and actin antibodies. A representative Western blot from UACC-893 cells is shown. Error bars refer to standard error of the mean of triplicate experiments (***$p<0.001$ by two-tailed unpaired student's t-test).

Figure 3: JAM-A regulates HER2 degradation and downstream AKT activation

(A) Western blot analysis of JAM-A, ER-α, HER2, p-AKT S473, total AKT, p-ERK and total ERK protein expression in MCF7-HER2 cells transfected for 72h with 25nM of control siRNA (siGENOME non-targeting siRNA #1, Dharmacon) or JAM-A siRNA (SASI_Hs01_00049785, Sigma-Aldrich, Poole, UK) using the Dharmafect-1 siRNA transfection system. Vehicle control (DMSO) or the proteosomal inhibitor MG132 (10μM) were present for the final 4h. Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A, HER2, ERα, p-AKT-S473 (Cell Signaling Technologies, MA, USA), AKT (Cell Signaling Technologies, MA, USA), p-ERK, ERK and actin antibodies. **(B)** Densitometric quantification of the effects of JAM-A knockdown alone (DMSO condition). HER2, ER-α and p-AKT protein levels were significantly reduced (*$p<0.05$, ***$p<0.001$ by two-tailed unpaired student's t-test). **(C)** Densitometric quantification of the effects of MG132 treatment relative to DMSO conditions. Protein levels of HER2, ER-α and p-AKT (reduced upon JAM-A knockdown) were partially restored following proteasomal inhibition. In all graphs, error bars refer to standard error of the mean of triplicate experiments.

Supplemental Figure S1: JAM-A immunohistochemical staining in breast cancer tissue microarrays

JAM-A immunohistochemistry (IHC) was performed as we have previously described (McSherry *et al.*, 2009) on 4μm sections of two separate formalin-fixed paraffin-embedded TMAs totaling respectively 48 and 167 invasive breast cancers. Membranous expression of JAM-A in tumor cells was scored 0, 1+, 2+ or 3+ based on staining intensity. JAM-A results were scored by four independent observers including one pathologist. Scale bar; 5μm.

Supplemental Figure S2: Western blot analysis of JAM-A, HER2 and phospho-HER2 expression across a range of breast cancer cell lines.

Western blot analysis of JAM-A, HER2, p Y877-phospho-HER2 and Y1221-phospho-HER2 protein expression in a range of cell lines expressing different profiles of the clinicopathologically-relevant molecular markers HER2 and ER-α. Cell extracts were separated by SDS–PAGE and immunoblotted with anti-human JAM-A, HER2-p-1221/1222, HER2-p-877 (Cell Signaling Technologies, MA, USA), HER2 and actin antibodies.

Figure 1

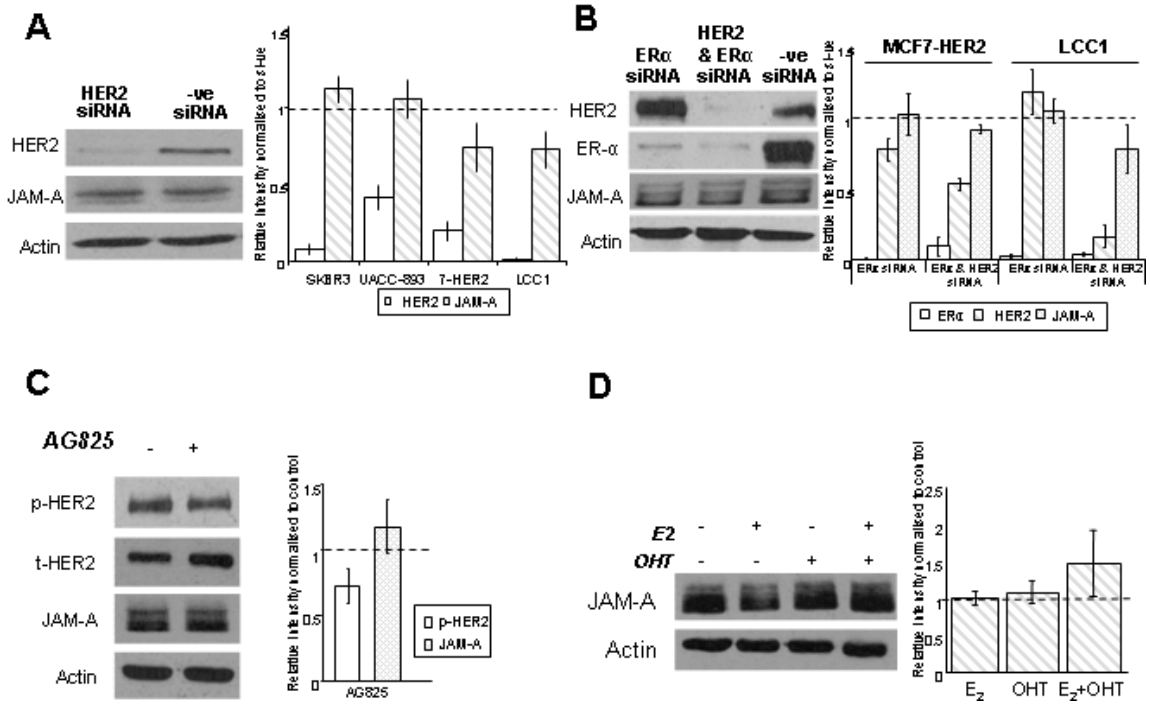


Figure 2

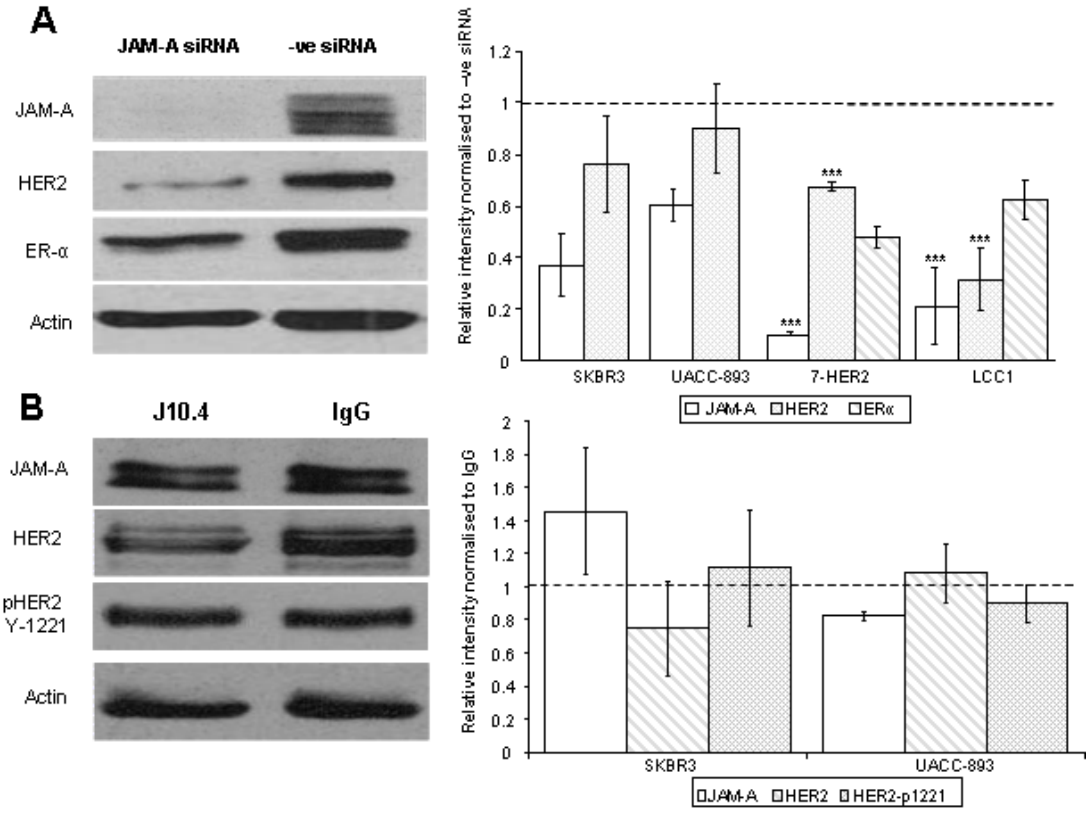
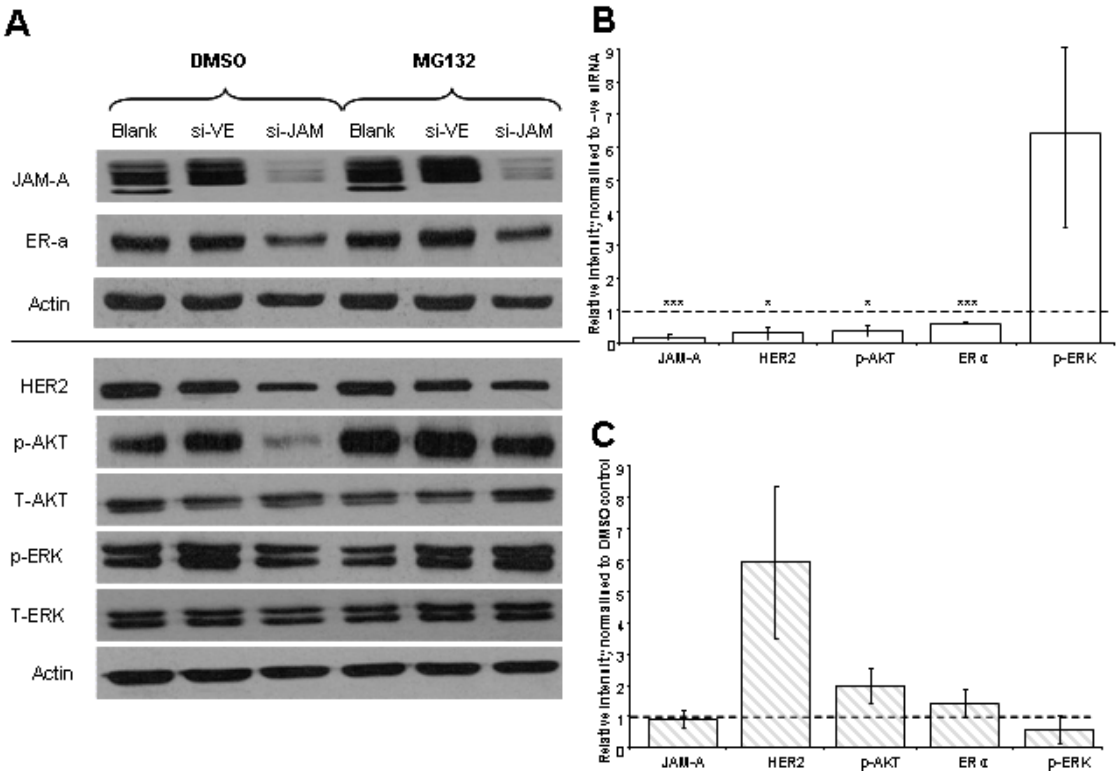
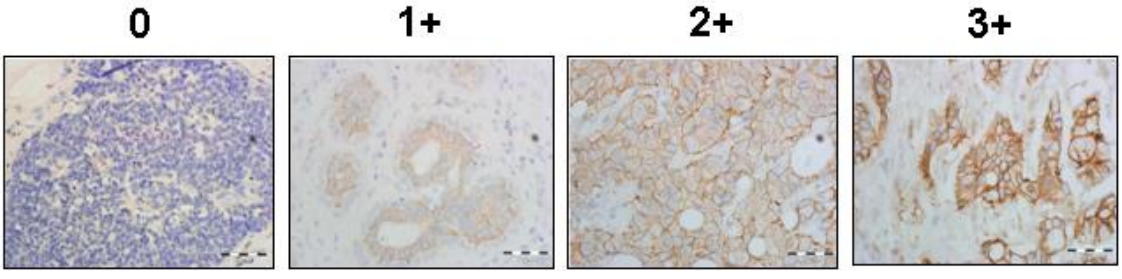


Figure 3



Supplemental Figure S1



Supplemental Figure S2

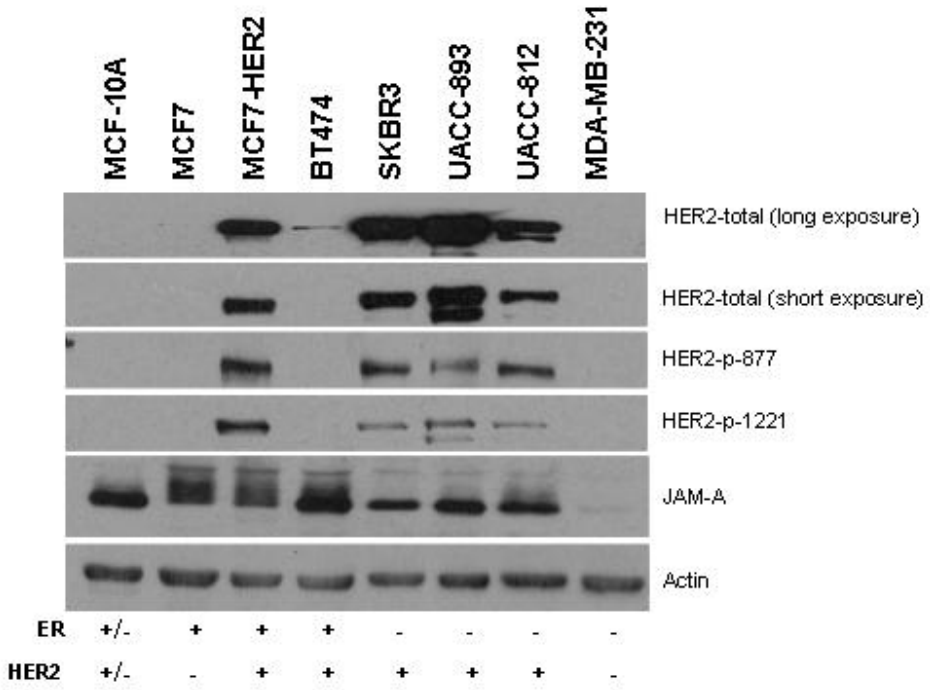


Table 1 - Clinicopathological Features of TMA#2 Stratified According to JAM-A Expression

Variable	N	Moderate/High-	Low JAM-A	P-value
Age (yrs)	166			
n		92	74	
Median (range)		53 (32-85)	53 (35-84)	
HER2 Status	150			**0.019***
0		30 (37.5)	39 (55.7)	
I-III		50 (62.5)	31 (44.3)	
Breast Cancer Subtype	122			**0.005****
Luminal A		18 (29)	31 (51.7)	
Luminal B		30 (48.4)	27 (45)	
HER2		9 (14.5)	1 (1.7)	
Basal		5 (8.1)	1 (1.7)	
Grade	118			**0.001****
1-2		27 (40.9)	37 (71.2)	
3		39 (59.1)	15 (28.8)	
ER Status	125			**0.001****
0		18 (27.7)	3 (5)	
I		47 (72.3)	57 (95)	

Values in parentheses indicate percentages within the JAM-A group,
* marks statistically significant values, where * $p<0.05$ ** $p<0.01$.
ER = Estrogen Receptor. PR = Progesterone Receptor.

Supplemental table S1 - Clinicopathological Features of TMA#1 Stratified According to JAM-A Expression

Variable	N	Moderate/High-	Low JAM-A	P-value
Age (yrs)	48			
n		20	28	
Median (range)		52.5 (28-75)	40.5 (18-82)	
Tumor size	48			**0.033***
Tis-T1		4 (20)	14 (50)	
T2-T3		16 (80)	14 (50)	
PR Status	47			**0.014***
I-II		20 (100)	20 (74.1)	
III		0 (0)	7 (25.9)	
ER Status	47			**0.073**
0		11 (55)	8 (29.6)	
I-III		9 (45)	19 (70.4)	
Breast Cancer Subtype	47			**0.023***
HER2+/ER-/PR- tumors		7 (35)	2 (7.4)	
Luminal and triple negative tumors		13 (65)	25 (92.6)	

Values in parentheses indicate percentages within the JAM-A group,

* marks statistically significant values, where *p<0.05

ER = Estrogen Receptor. PR = Progesterone Receptor. TNM = Tumour size, regional lymph Node status and distant Metastasis.