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Genomic and Proteomic Pathway Mapping Reveals Signatures of Mesenchymal-Epithelial Plasticity in Inflammatory Breast Cancer

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1. Introduction

1.1 Inflammatory breast cancer as a distinct clinicopathologic entity

There are several clinically distinct types of breast cancer, which include early stage breast cancer, locally advanced breast cancer (LABC) and metastatic breast cancer. The most rare but lethal form of LABC is inflammatory breast cancer (IBC) (reviewed in 1). This type of breast cancer accounts for an estimated 2- 5% of all breast cancers in the United States and up to 20% of all breast cancers globally (2-4). Although primary IBC is less commonly diagnosed than other types of breast cancer, IBC is responsible for a disproportionate number of breast cancer-related deaths that occur each year world-wide due to its propensity to rapidly metastasize. (2-4). Women diagnosed with IBC have a significantly shorter median survival time (~ 2.9 years) than women with either LABC (~ 6.4 years) or non-LABC breast cancer (>10 years). The clinical diagnosis of IBC is based on the combination of the physical appearance of the affected breast, a careful medical history, physical examination, and pathological findings from a skin biopsy and/or needle or core

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biopsy to confirm the diagnosis of carcinoma. The symptoms of IBC include a rapid onset of changes in the skin overlying the involved breast, including edema, redness and swelling involving over one half to two thirds of the breast, which may include a wrinkled, orange peel appearance in the skin, defined as "peau d'orange" (5,6). IBC is diagnosed in women at a younger age and since it does not present as a lump but rather occurs as sheets or nests of cells defined as tumor emboli, IBC is difficult to detect using mammography and requires more sophisticated imaging modalities such as magnetic resonance imaging (MRI) and positron emission tomography (PET) (1, 7). Since IBC occurs more rarely than other variants of breast cancer, neither the general public nor primary care physicians are aware of the signs and symptoms of IBC. It is commonly misdiagnosed as an infection, such as mastitis, resulting in delays in initiation of appropriate treatment. Disease progression is very rapid in IBC patients, with symptoms appearing often within days or weeks, and IBC patients commonly have lymph node metastasis at the time of first accurate diagnosis (6).

The skin changes observed in the involved breast of IBC patients are the first clinical signs of IBC and are believed to be associated with the presence of tumor cells that tightly aggregate to form multi-cellular nests of cells, defined as tumor emboli, that invade into the dermis. These tumor emboli are one of the classical histopathological findings in IBC (8, 9). Although the presence of dermal tumor emboli is not a requirement for a diagnosis of IBC, approximately 75% of IBC patients have tumor emboli that are observed in skin punch biopsy tissue and they serve as one of most distinctive characteristic signatures of IBC.

1.2 Models of inflammatory breast cancer

Historically, one of the barriers in research into the mechanisms underlying the aggressive metastasis of IBC has been the lack of sufficient numbers of cell lines and pre-clinical animal models derived from IBC patients with diverse breast cancer subtypes. Although there are *in vitro* and *in vivo* models available for a number of IBC cell lines including SUM149 (10-13), SUM190 (10-13), KPL-4 (14) and MDA-IBC-3 (15) with the associated animal xenograft models, the majority of IBC research has primarily used the SUM149 cell system as a model of IBC. Studies described in this chapter use all available IBC cell lines and animal models, including the only animal model of IBC that recapitulates formation of tumor emboli, Mary-X (16, 17).

When Mary-X tumor cells are propagated *in vitro,* they exhibit the unique characteristic of only existing as tightly adhered cell aggregates that we have defined as tumor spheroids (Figure 1 A). As such, Mary-X tumor spheroids provide a convenient *in vitro* surrogate for IBC tumor emboli that form *in vivo*. When Mary-X tumor spheroids are serially transplanted by subcutaneous injection into female immunocompromised mice, primary Mary-X tumors develop (Figure 1 B). In addition, aggregates of cells bud off from the primary tumor and form local metastatic lesions that appear as tumor emboli that invade into the dermal tissue (Figure 1 C). Mary-X also forms distant metastasis at multiple sites, including the lung (Figure 1 D). Triple color immunofluorescence studies demonstrate the highly proliferative characteristic of Mary-X tumor emboli, as defined by Ki-67 staining (Figure 1 E). These tumor emboli invade into and are encircled by lymphatic endothelium within the dermis, defined by their selective expression of podoplanin, a marker specific for lymphatic endothelial cells (Figure 1 E) (16,17). This is visual evidence of the propensity of IBC tumor emboli to exhibit cohesive invasion and to metastasize locally into the dermal lymphatic vessels, which may be one of the mechanisms underlying the common lymph node metastasis that occurs in IBC patients at the time of first diagnosis.
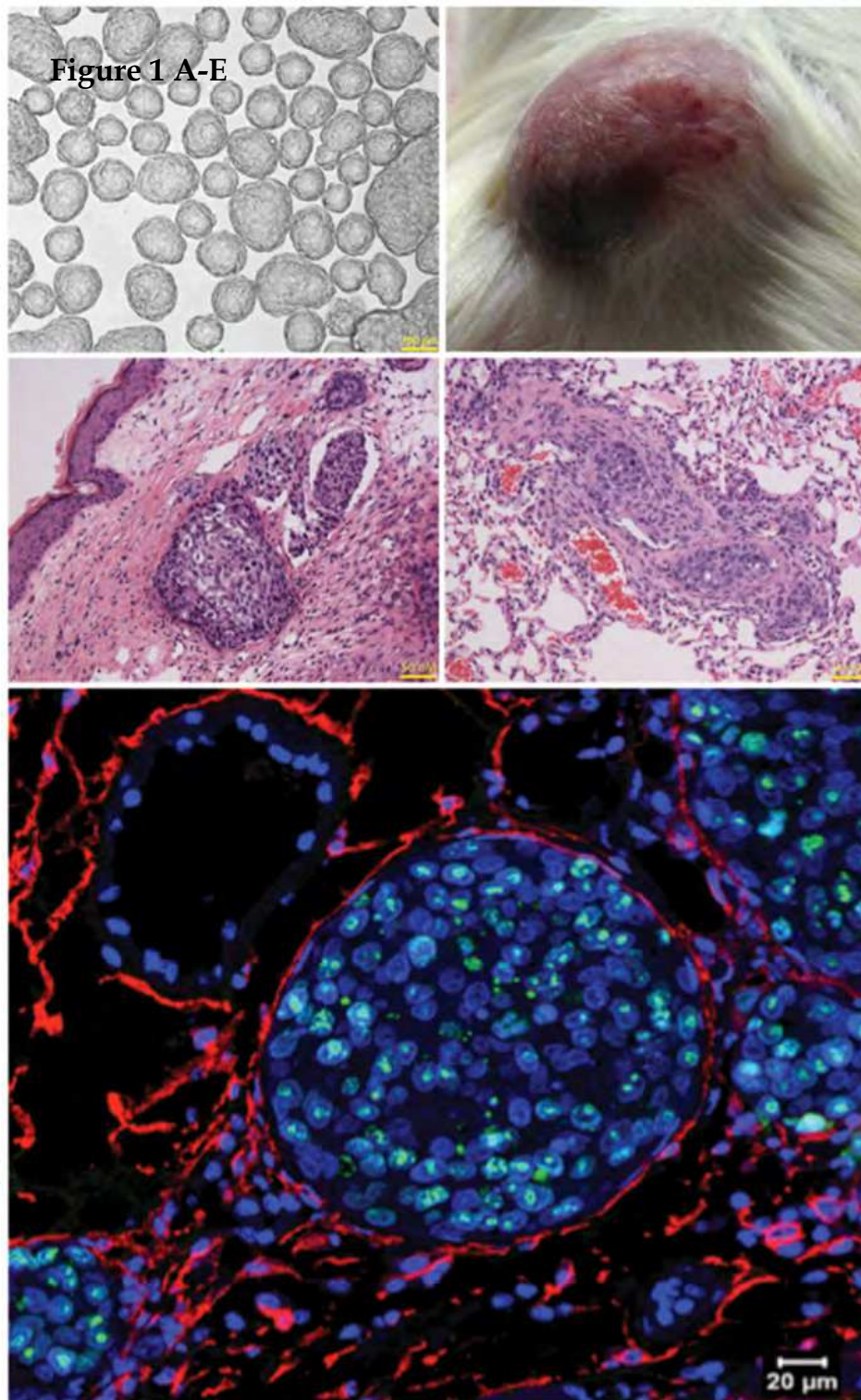
Fig. 1. **A.** Mary-X tumor spheroids can be propagated *in vitro* only as tightly adhered cell aggregates. **B.** Light micrograph image of a primary Mary-X tumor visible as a tumor with redness of the skin following subcutaneous injection of Mary-X spheroids into immunocompromised mice.**C.** Light micrograph of tissue section isolated from a mouse bearing a Mary X primary tumor that has formed local metastases that are visible in dermal tissue as tumor emboli. **D.** Light micrograph of Mary-X tumor emboli that have formed

metastastic lesion in the lung. **E**. Image of Mary-X tumor tissue with highly proliferative Mary-X tumor emboli, as determined by staining with Ki-67 (green fluorescence), that are encircled by lymphovascular endothelium, defined by their selective staining with anti-podoplanin antibodies (red fluorescence), demonstrating their propensity to invade as a cohesive unit of aggregated cells into the dermal lymphatic vessels.

The most recent study that examined the predominant subtypes of inflammatory breast cancers reported that there are approximately equal percentages of basal-like, Her2 amplified and normal like subtypes among IBC patients, with a slightly higher percentage of IBC tumors that are of the luminal B subtype (18). Interestingly, the models of IBC developed thus far are either triple negative basal like, such as the Mary-X and SUM149 IBC cells or are of the luminal B subtypes, such as SUM190 and MDA-IBC-3 cell lines. Based on transcriptional analysis and hierarchical clustering, SUM190, MDA-IBC-3 and KPL-4 cells have characteristics most closely associated with the luminal B subtype and are positive for at least one of the hormone receptors and the Her2 oncogene.

For studies described in this chapter, we examined the SUM149, SUM190, KPL-4, MDA-IBC-3 cell lines and Mary-X tumor spheroids as *in vitro* models of IBC and we also evaluated xenograft tissues generated from animals bearing each of these cell lines/cell systems. This represents the most comprehensive analysis of all available IBC cell lines and animal systems to date.

1.3 E-cadherin as a signature of inflammatory breast cancer

One of the only well characterized histological markers of IBC tumor emboli is their robust expression of the E-cadherin (19-22). In general, the classic cadherins, including E-cadherin, N-cadherin and P-cadherin are transmembrane glycoproteins that are linked to actin cytoskeletal networks and other cytoplasmic and transmembrane proteins by forming complexes with the catenins including α-catenin/vinculin, β-catenin, junction plakoglobin (JUP)/γ–catenin, and p120/δ catenin (23, 24). E-cadherin is considered to be a predominant regulator of what has been defined as "collective cell interactions" (25). Therefore, E-cadherin mediates tight cell:cell homophilic interactions exhibited by epithelial cells (reviewed in 26). Using the Mary-X model of IBC, E-cadherin antibodies were found to induce the loss of integrity of Mary-X spheroids and when injected via the intravenous route into mice bearing Mary-X tumors with known pulmonary metastasis, the metastatic lesions were diminished (16, 17). Additional evidence for the critical role of E-cadherin to survival to tumor emboli came from studies in which Mary-X spheroids containing a dominant-negative E-cadherin mutant (H-2K(d)-E-cad) which lacked the extracellular binding domain but retained the β-catenin binding domain exhibited loss of integrity of the Mary-X tumor spheroids due to inhibition of the tight cell:cell interactions. When injected into mice, these Mary-X tumor spheroids containing dominant-negative mutant constructs were only weakly tumorigenic and inhibited the ability of Mary-X cells to form tumor emboli (16, 17). Using the SUM149 IBC cell line, other studies demonstrated that the presence of dominant negative E-cadherin (H-2kd-E-cad) cDNA blocked SUM149 invasion *in vitro*, which was associated with a decreased expression of the matrix metalloprotease enzymes (27). Recent studies demonstrating that blockade of p120/δ catenin, which anchors E-cadherin within the plasma membrane or inhibition of the translation initiation factor eIF4GI, which regulates translation of specific mRNAs such as p120/δ catenin, resulted in loss of integrity of SUM149 tumor spheroids (28). Taken together, these studies suggest that E-cadherin is

critical to the invasive and metastatic phenotype of IBC tumor emboli, and also indicate that E-cadherin and p120/δ catenin may act in concert to maintain the integrity of the tightly aggregated tumor cells that comprise the IBC tumor emboli. These studies suggest that E-cadherin may function not only as part of the signature of IBC but may also serve as a therapeutic target which, when effectively blocked, results in inhibition of the tight cell:cell aggregation of IBC tumor spheroids *in vitro* and abrogates the metastatic potential of IBC tumor emboli *in vivo*.

1.4 Linking E-cadherin as a signature of inflammatory breast cancer and the process of the epithelial mesenchymal transition (EMT) in metastasis

While IBC is a variant of breast cancer that exhibits a program of accelerated metastasis, the robust expression of E-cadherin by aggregates of cells within IBC tumor emboli in patients' tissues and in pre-clinical models of IBC is, at least on first examination, paradoxical to the current hypothesis that the initiation of metastasis occurs through a specific process defined as the epithelial mesenchymal transition (EMT). EMT and the reverse process of mesenchymal epithelial transition (MET) are interlinked programs that are essential to normal embryonic development, as well as to appropriate wound healing and tissue regeneration following injury (29, 30). In these settings, the reversible processes of EMT and MET confer the ability of cells to exhibit plasticity in both their morphology and function (29). In the setting of embryonic development, EMT and MET are highly organized and precisely regulated programs that are critical to appropriate formation of the epithelial, mesoderm and endodermal layers required for organ formation (29). The process of EMT is reactivated as a developmental program in response to injury; as an example, an EMT process is induced in epithelial keratinocytes of surface epithelium at the leading edge of a wound. In this case, the epithelial cells have an intermediate "metastable" phenotype, and acquire an elongated mesenchymal morphology, increase their migratory activity while remaining attached to each other until closure of the wounded area is accomplished (29, 30). In a tumor setting, the process of EMT includes a number of functional changes in tumor cells which include activation of transcription factors including *ZEB1* and *ZEB2*, *TWIST1*, *SNAIL*, *SLUG*, with the associated loss of expression of specific cell-surface proteins that regulate the epithelial phenotype including E-cadherin and zona occludins-1. In addition, there is a concomitant gain of other genes that regulate the mesenchymal phenotype such as N-cadherin, and reorganization and expression of cytoskeletal proteins such as vimentin and alpha smooth muscle actin, production of enzymes that degrade extracellular matrix such as matrix metalloproteinase 2 (MMP-2), also known as gelatinase, and expression or suppression of specific miR families (31). There are several other changes in function of tumor cells undergoing the process of EMT including the acquisition of characteristics that are similar to stem cells including expression of surface markers CD44+/CD24$^{-/low}$ (31, 32).

While IBC is the variant of breast cancer that exhibits the most accelerated metastasis, and has been characterized as being enriched for cells expressing markers of tumor initiating cells/cancer stem cells, including expressing CD44$^+$/CD24$^{-/low}$, aldehyde dehydrogenase 1 (ALDH-1$^+$), and CD133+ (33-35), the robust expression of E-cadherin by IBC tumor emboli is inconsistent with the current hypothesis that initiation of metastatic progression occurs through the process of EMT. This chapter will highlight our studies that have used whole unbiased transcriptional analysis and broad-scale protein pathway activation mapping to define the specific patterns of expression of genes, proteins and miRs, along with functional

protein signaling architecture that collectively provide insight into the distinct signature of IBC. It is the changes in the molecular machinery that define the extreme plasticity and collective tumor cell migration patterns exhibited by IBC tumor cells and tumor emboli that are the metastatic lesion of this lethal variant of breast cancer.

2. Defining the signatures of inflammatory breast cancer

2.1 Whole transcriptome analysis and validation of gene signature in IBC cell lines and tumor emboli

Affymetrix microarrays were used to evaluate 56,000+ probe sets expressed by all currently available IBC cell lines and cell systems including SUM149 and Mary-X tumor spheroids which are of the triple negative subtype and the SUM190, MDA-IBC-3, KPL-4, which are of the luminal B molecular subtype. The non-IBC cell lines included in the analysis were MDA-MB-231, SUM159, and MCF-7 human breast cancer cell lines. The MDA-MB-231 and SUM159 cells are both classified as triple negative breast cancer cell lines and MCF-7 cells are of the luminal A molecular subtype.

Whole unbiased transcriptome analysis revealed that, regardless of molecular subtype, the IBC cell lines expressed *CDH1*, which encodes for E-cadherin, compared to non-IBC breast cancer cell lines, with the exception of MCF-7 cells (Figure 2). There was heterogeneity in *CDH1* expression by the different IBC cell lines, with KPL-4 cells having the lowest level of *CDH1* expression. In addition to *CDH1*, IBC cell lines expressed other genes that have previously been shown to be involved with regulating tight cell:cell adhesion of epithelial cells through formation of the adherens junctions including *DSC2*, which encodes for desmocollin 2, and *JUP*/γ catenin and the expression of these two genes by the individual IBC cell lines mirrored that of *CDH1*. Although gene expression of *CTNNA1* and *CTNNB1*, which encode for α catenin and β catenin, respectively, was detectable in IBC cell lines, these genes were not differentially expressed at higher levels by IBC cell lines compared to non-IBC cell lines. One significant difference in the whole transcriptome analysis of IBC cell lines compared to non-IBC cell lines was the striking lack of expression of the zinc finger E-box binding homeobox 1 (*ZEB1*) transcription factor, also previously defined as transcription factor 8 (*TCF8*) and *ZFHX1A* (Figure 2). Analysis of other transcription factors related to the process of EMT revealed that *SNAI2*, which encodes for Slug protein, was expressed by all of the basal like breast cancer cells including SUM149, Mary-X, SUM159 and MDA-MB-231 but was not expressed by SUM190, which are luminal B, suggesting that gene expression of this transcription factor may be subtype dependent. Expression of other transcription factors including *ZEB2* was detectable however the pattern of expression did not appear to be related to molecular subtype or whether cells were IBC or non-IBC.

To validate the results of these transcriptome studies, tissues isolated from mice bearing Mary-X xenografts were assessed for the presence of emboli in tissue sections stained with hematoxylin and eosin (H&E) and the presence of tumor emboli in the dermis was noted (Figure 3 A). Serial sections of this same tissue isolated from Mary-X xenograft were stained with specific antibodies that identified E-cadherin expressed by tumor emboli in the dermis (Figure 3 B). A higher magnification light micrographic image demonstrates the presence of abundant E-cadherin protein on the surface of cells within the Mary-X tumor emboli in the dermis of the skin (Figure 3 C). Figure 3 D shows a micrometastastic lesion of Mary-X within lung tissue stained with E-cadherin antibodies, demonstrating that metastatic lesions of Mary-X have persistant expression of E-cadherin. Triple color immunofluorescence and

microscopy defined the specific patterns of co-localization of E-cadherin and JUP/γ catenin (Figure 3 E) in tissue sections of skin isolated from mice bearing Mary-X xenografts containing IBC tumor emboli within the dermis. E-cadherin and JUP/γ catenin both co-localized primarily to the plasma membrane of tumor cells within Mary-X tumor emboli (Figure 3 E). These results are the first to associate the expression of *CDH1*, which encodes for the transmembrane glycoprotein E-cadherin, by Mary-X tumor emboli, with increased expression of other genes, including *JUP*/γ catenin, that collectively regulate tight cell:cell homotypic aggregation by IBC tumor emboli. These results suggest that the upregulation of this specific cassette of genes is part of the distinct signature of IBC tumor emboli that are the local metastatic lesions of IBC.
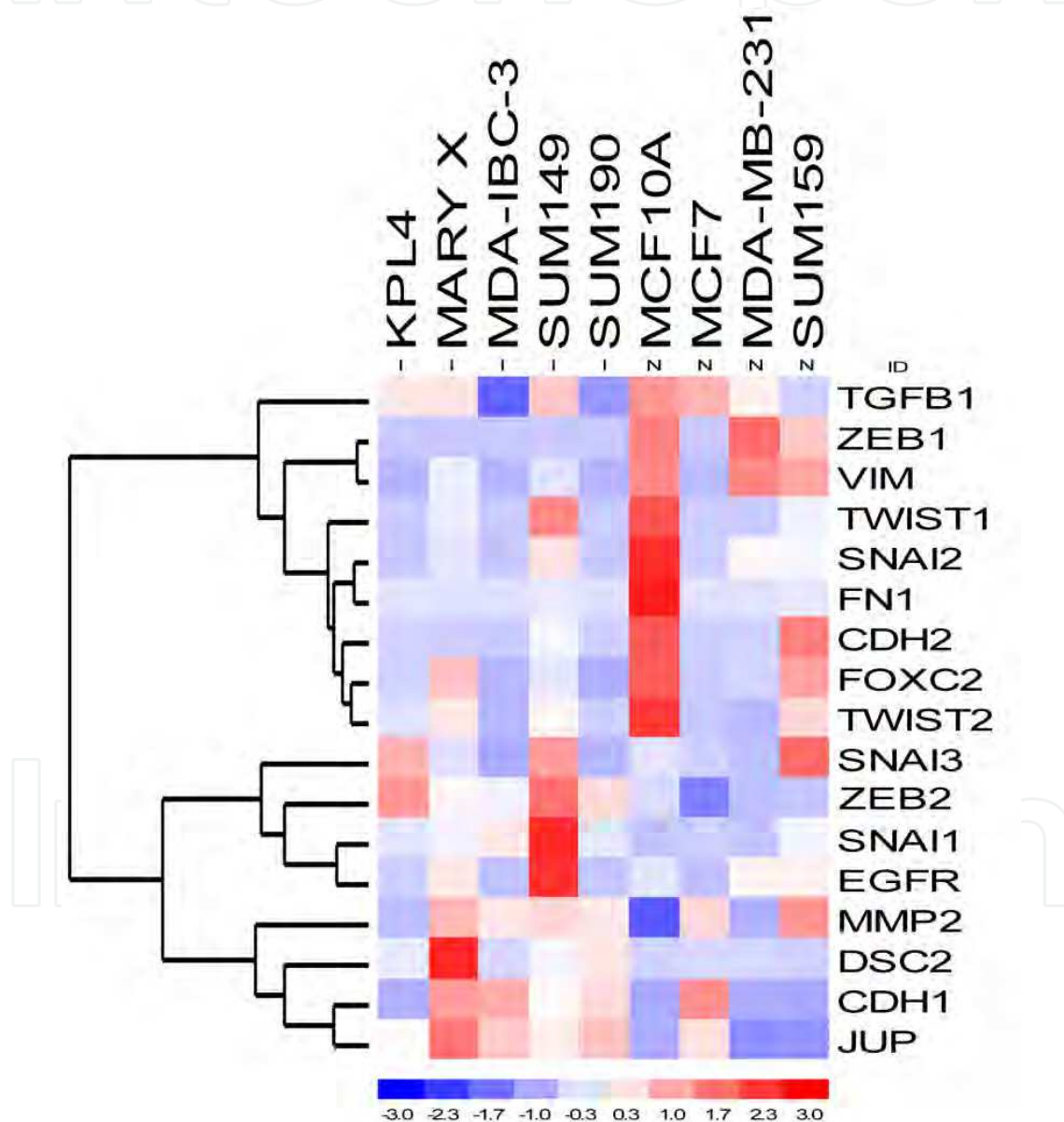

Fig. 2. Heatmap showing results whole unbiased transcriptome analysis of gene signatures of IBC cell lines compared to non-IBC cell lines revealed that IBC cell lines expressed high levels of *CDH1*, JUP/γ, and DCS2, with a lack of expression of *ZEB1* compared to non-IBC breast cancer cell lines.
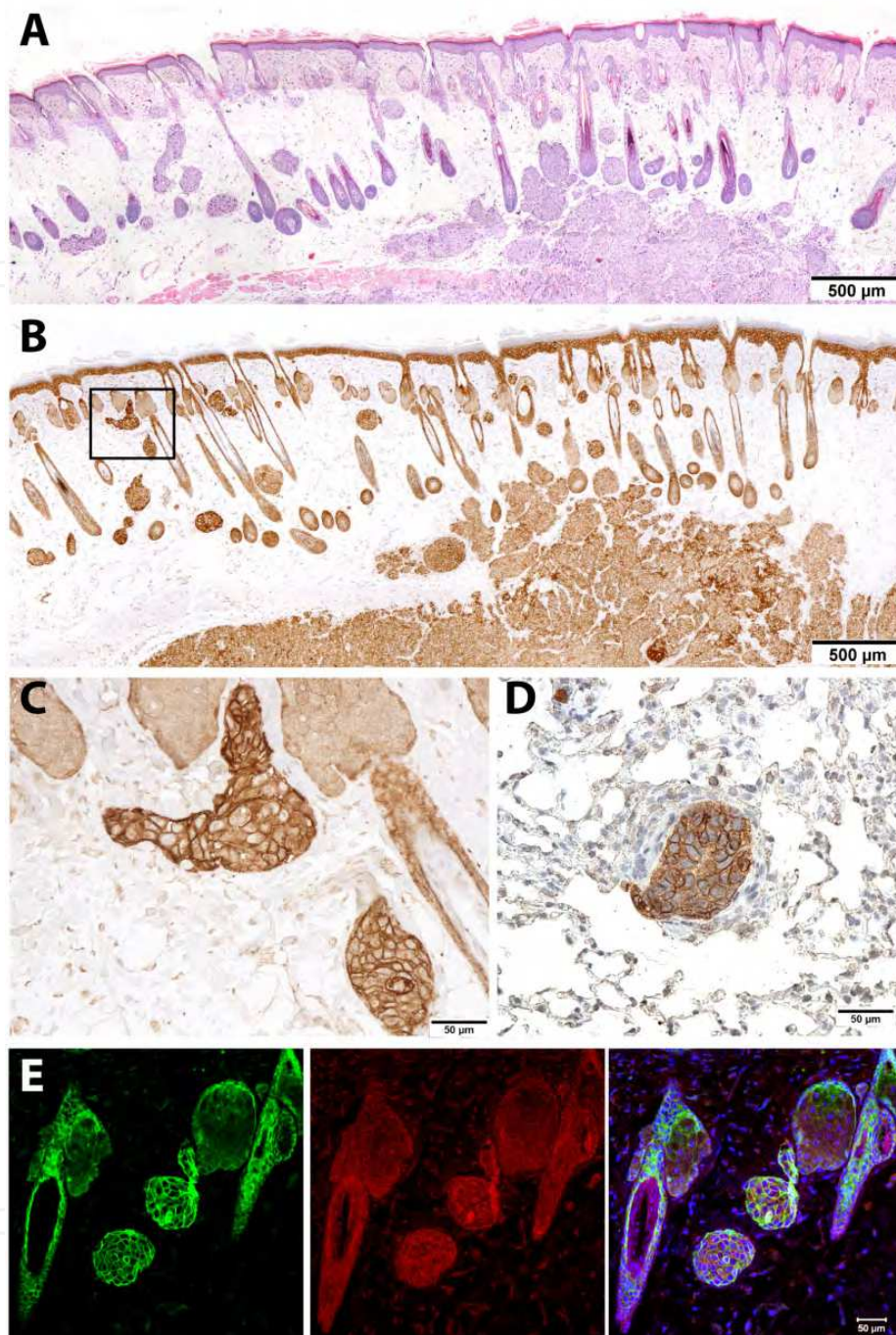
Fig. 3. **A**. H&E stained tissue section isolated from Mary-X xenograft demonstrating the presence of numerous tumor emboli within the dermis of the skin (4x magnification). **B and C.** Light micrographs of a serial section of tissue isolated from Mary-X xenograft as shown in Figure 3 A, stained with E-cadherin antibodies demonstrating that Mary-X primary tumor as well as numerous tumor emboli throughout the dermis express E-cadherin protein (Figure B. 4 x magnification and Figure C. 20X magnification). **D.** Light micrograph of section of lung tissue stained with E-cadherin antibodies demonstrating the presence of E-cadherin in pulmonary micrometastasis lesion (20x magnification). **E.** Triple color immunofluorescence and fluorescence microscopy defined the specific patterns of co-localization of E-cadherin and JUP/γ catenin at the surface of the plasma membrane of Mary-X tumor emboli.

2.2 MicroRNA signatures of inflammatory breast cancer cells

Differential expression of specific microRNAs (miRs) expressed by IBC cell lines compared to non-IBC cell lines was evaluated using a Human Cancer focused PCR array based miRNA analysis (SA Biosciences/Qiagen, Frederick, MD) and were validated by real time PCR. The specific miR identified as being differentially expressed by IBC cell lines was miR200c (Figure 4). The significance of the high expression of miR 200c lies in its reported function as an indirect transcriptional regulator of *CDH1* by *ZEB1/2*. Recent studies report that the reciprocal relationship between *ZEB1/2* and members of the miR 200 family is responsible for the switch between epithelial and mesenchymal states and is driven, in part, by an active autocrine TGF beta signaling network (36). The identification of miR200c as the primary miR expressed by IBC cell lines is consistent with previous studies demonstrating the reciprocal repression of E-cadherin by *ZEB1* through downregulation of miR 200c (37-41). Interestingly, the expression of miR 200c as the primary miRs in IBC cell lines provides independent validation of the observations that IBC cell lines are characterized by a specific gene signature that includes expression of CDH1 and other genes associated with homotypic aggregation and tight cell:cell adhesion, with a lack of expression of *ZEB1* identified using whole transcriptome analysis.
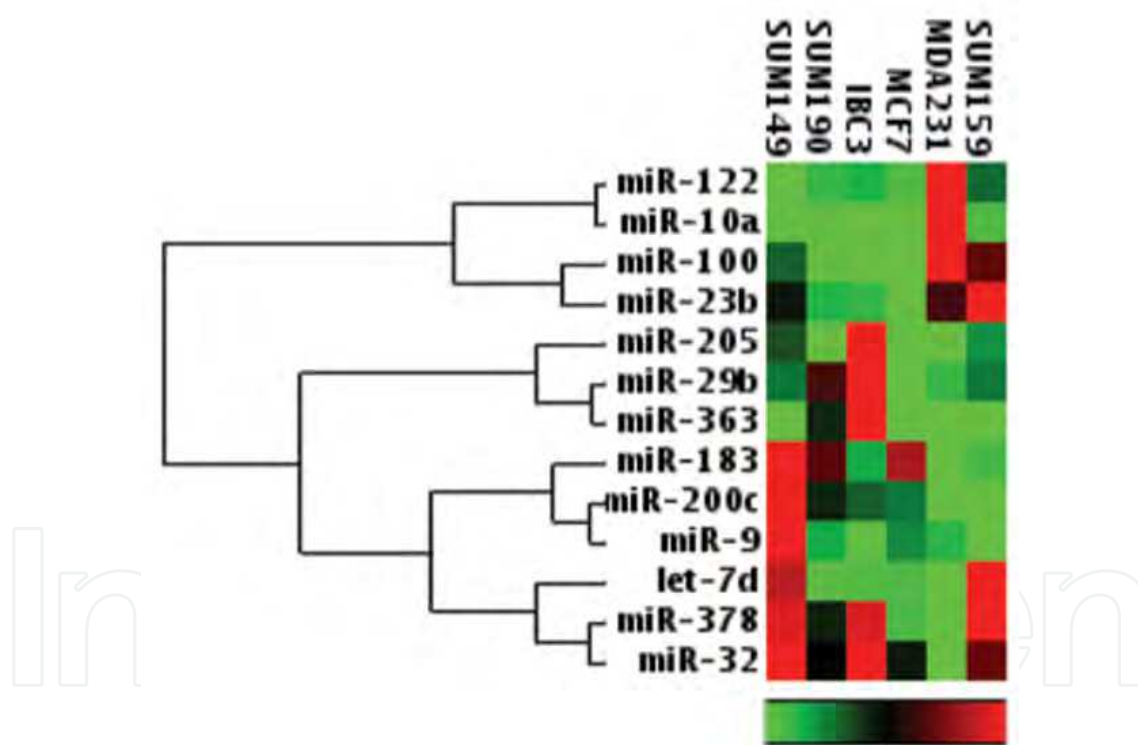

Fig. 4. Heatmap of miRs in SUM149 and SUM190 cells compared to non-IBC cell lines. Analysis of abundantly expressed miRs revealed that IBC cell lines express high levels of miR 200c.

Although the expression of miR 200c in tandem with the robust expression of a cassette of genes associated with homotypic aggregation including *CDH1*, *DSC2*, and *JUP* appears to be paradoxical to the current understanding of the process of metastatic progression associated with the alternations that occur during EMT, previous studies reported that multi-cellular tumor cell clusters are more efficient at formation of metastasis compared to

single cells (42). Moreover, studies that examined primary breast tumors and the corresponding liver, lung and brain metastasis revealed increased E-cadherin expression in the metastatic lesions compared to the primary tumors (43). As an example, the MDA-MB-231 triple negative breast cancer cells have a mesenchymal phenotype and do not express E-cadherin, were demonstrated to re-express E-cadherin protein in spontaneous MDA-MB-231 derived metastatic foci, supporting the hypothesis that the reversion from EMT to exhibit characteristics of the process of MET occurs at sites of metastasis distant from a primary tumor that may exhibit an EMT phenotype (43). Collectively, these studies suggest that IBC, as the most lethal variant of breast cancer, exhibits signatures that point to an ongoing process of MET, which is consistent with the ability of tumor emboli to survive, to undergo what has been defined as "cohesive invasion" and to rapidly colonize organs and tissues distant from the primary tumor (42-44).

2.3 Proteomic pathway mapping of IBC cell lines

Reverse phase microarray (RPMA) technology was developed by our laboratory to address the challenges associated with other types of protein assays, namely the ability to quantitatively measure the levels and activation/phosphorylation state of key signaling proteins in a multiplexed fashion using microscopic quantities of tumor tissue and cells (45-48). We used RPMA in the present studies to identify the specific signal transduction pathways and molecules activated in IBC cell lines compared with non-IBC cell lines. The results of protein pathway mapping identified significant activation of specific pathways in IBC cells including E-cadherin (p>0.001) (Figure 5 A) and phospho-focal adhesion kinase (FAK) at Y576/577 (p>0.015) (Figure 5 B). Interestingly, recent studies have demonstrated that blocking FAK results in down regulation of the cell:cell adhesion properties of E-cadherin (49). Additionally, histone deacetylase (HDAC) inhibitors have been reported to inhibit FAK protein expression (50). These results provide independent validation of the observations from the whole transcriptome analysis identifying E-cadherin expression as a primary characteristic of IBC and suggests that specific therapeutic molecules, such as HDAC inhibitors that can block the functions of E-cadherin and FAK, may be useful in targeting IBC tumor emboli.

3. Signature based therapeutic targets in inflammatory breast cancer

The whole transcriptome based analysis identified E-cadherin, DSC2, and JUP/γ catenin as gene signatures of the tight cell:cell adhesion exhibited by IBC cells and tumor spheroids. Taken together with the proteomic-based identification of E-cadherin and FAK, these results suggest that these are IBC specific targets appropriate for the activity of HDAC inhibitors. We therefore evaluated the effects of this class of agents on IBC tumor spheroids, which provide an *in vitro* surrogate for IBC tumor emboli. Using fluorescence microscopy, wee demonstrate that the HDAC inhibitor, Suberoylanilide Hydroxamic Acid (SAHA;Vorinostat® Merck, Inc), destroys the integrity of Mary-X tumor spheroids and induce apoptosis as determined by TUNEL staining (Figure 6 A). In addition, SAHA induced the translocation of E-cadherin and JUP/γ catenin from the plasma membrane to the cytoplasm of Mary-X tumor spheroids, resulting in a loss of integrity of the tumor spheroids (Figure 6 B). Using SUM149 IBC tumor spheroids, we previously reported that SAHA induced a loss of integrity and viability of SUM149 tumor spheroids through translocation of E-cadherin protein from the plasma membrane to the cytoplasmic

compartment, without altering the amount total E-cadherin protein, suggesting a change in functional activity of E-cadherin (51). We also found that SAHA induced a loss of the tight
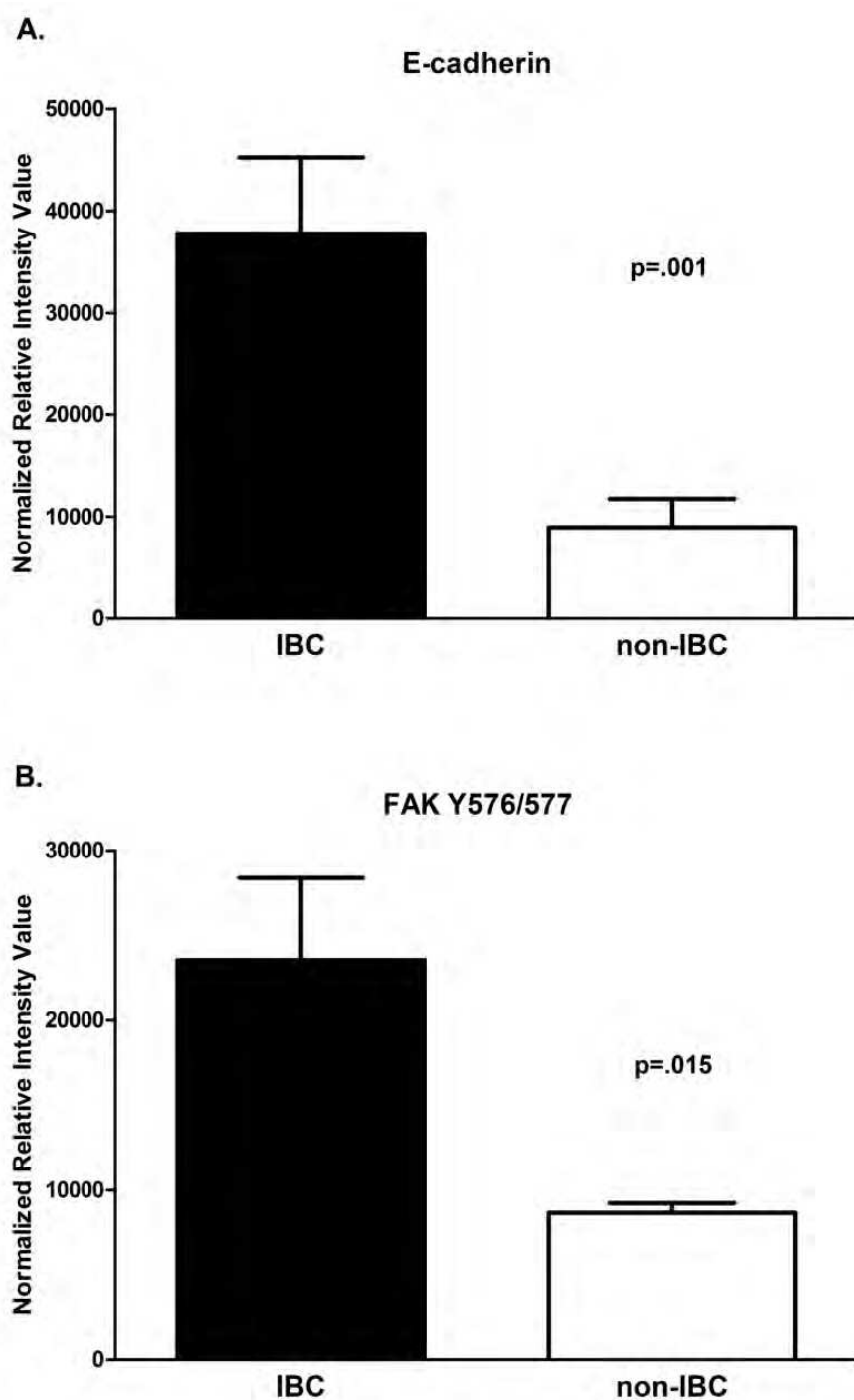

Fig. 5. **A.** RPMA analysis demonstrates significant increase in E-cadherin protein in IBC tumor spheroids including Mary-X, SUM149, SUM190 and MDA-IBC-3 compared to non-IBC cells MDA-MB-231 and SUM159. **B**. RPMA analysis revealed first time evidence for activation of focal adhesion kinase (FAK) protein at Y576/577. Histograms of total E-cadherin and phosphorylated FAK at Y397 and Y576/577 and are shown for both IBC cell lines and non-IBC cell lines (Standard deviations are shown with p values).
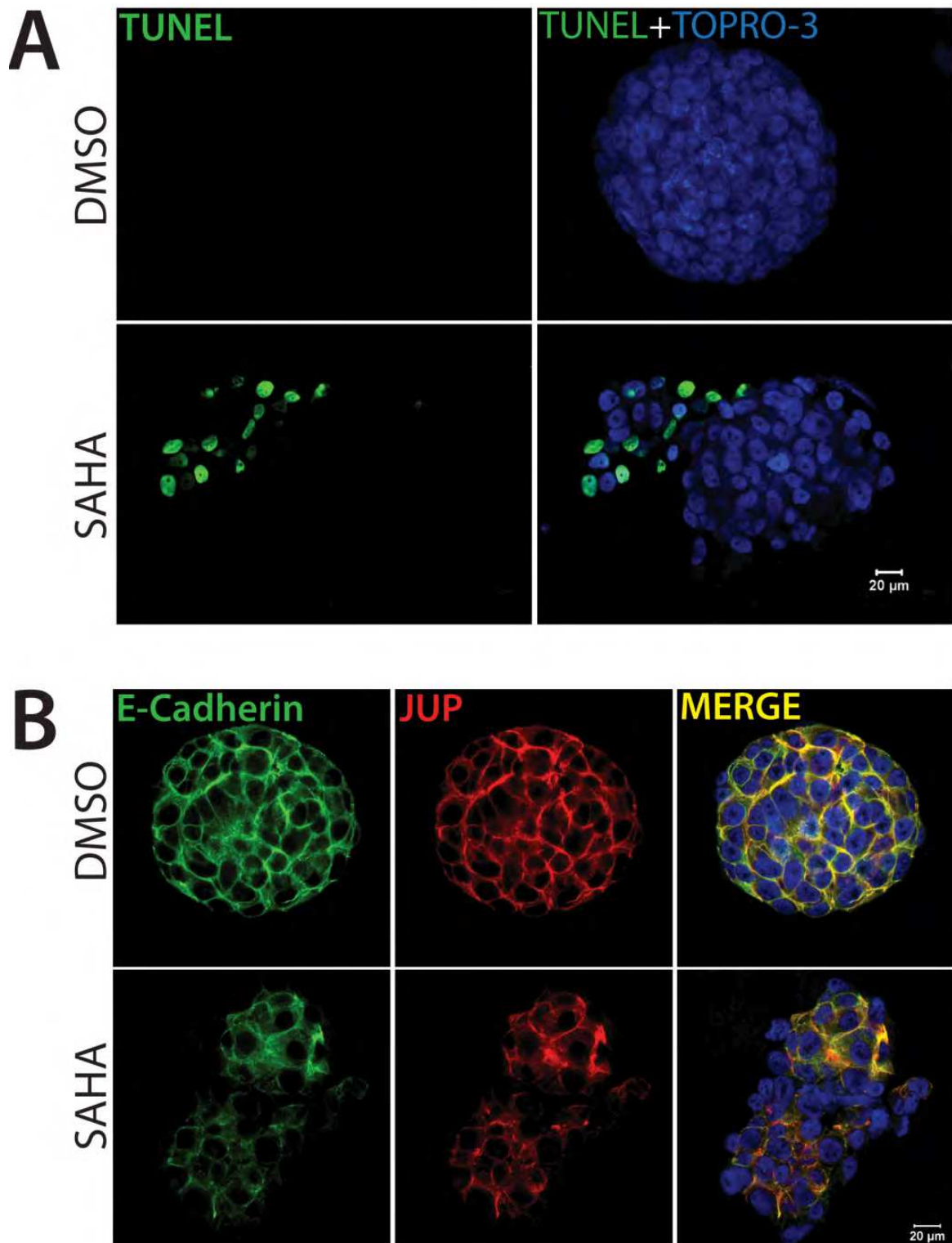
Fig. 6. **A.** Fluorescence microscopy revealed that Suberoylanilide Hydroxamic Acid (SAHA;Vorinostat® Merck, Inc) induced apoptosis in Mary-X tumor spheroids as evaluated by analysis of TUNEL staining. **B.** Flourescence microscopy demonstrated that SAHA induced translocation of E-cadherin (green fluorescence) and JUP/γ catenin (red fluorescence) from the plasma membrane of Mary-X tumor spheroids to reside primarily within the nucleus, resulting in a loss of integrity of the tumor spheroids.

cell: cell aggregation mediated by E-cadherin, resulting in the inhibition of self renewal and clonogenicity of SUM149 tumor spheroids as well as inhibited the tight aggregation of freshly isolated IBC patient tumor cells derived from pleural effusion (51). In non-IBC cell lines, SAHA induces apoptosis and in E-cadherin null cells, SAHA can re-induce E-cadherin, thus reversing EMT. Collectively, these are the first studies to identify the HDAC inhibitors as a class of therapeutic agents that abrogate the functional role of E-cadherin in formation of adherens junctions in IBC tumor spheroids that leads to destruction of these 3 dimensional multi-cellular structures which are surrogates for IBC tumor emboli as the metastatic lesions of this lethal variant of breast cancer. The present results suggest that proteins encoded by the cassette of genes that serve as part of the signature of IBC tumor emboli which specifically regulate the tight cell:cell adhesion of cells within IBC tumor emboli, including E-cadherin, DSC2 and JUP/γ catenin, represent potential therapeutic targets for eliminating IBC tumor emboli. In addition, these studies suggest that HDAC inhibitors are a class of compounds that effectively target the IBC tumor emboli for destruction. Studies are ongoing to determine the potential of HDAC inhibitors for their clinical utility.

4. Summary and conclusions

This chapter provides an overview of newly described IBC-specific molecular alterations expressed in IBC cell lines, tumor spheroids and tumor emboli characterized by a unique plasticity of this distinct variant of breast cancer. The concomitant use of gene and miR expression profiling as well as functional protein pathway activation mapping provides an unprecedented molecular/systems-level view of IBC. While IBC cells, tumor spheroids and tumor emboli express abundant levels of E-cadherin that is expressed in concert with other genes that collectively mediate tight homotypic aggregation of IBC tumor cells, with a loss of *ZEB1*, and express miR 200c, a repressor of *ZEB1*, which is consistent with the process of MET, IBC cells simultaneously express transcription factors that support invasion and metastasis, characteristic of the process of EMT. Proteomic analysis of the signaling architecture of IBC reinforces and expands on the genomic findings of activation of signaling pathways specific to IBC, validating the central role of E-cadherin to IBC tumor emboli. Our observations suggest that, as the most aggressive variant of breast cancer, IBC retains an epithelial phenotype characterized by cell:cell aggregation and cohesive invasion (32), and exhibit a program of accelerated metastasis by IBC tumor emboli distinguished by expression of specific genes, miRs and signaling proteins. The specific function of the genes within this distinct signature of IBC plasticity, which include genes involved in the processes of both MET, such as E-cadherin and FAK activation that may mediate the cohesive invasion of tumor emboli, with lack of *ZEB1*, while simultaneously expressing genes associated with EMT, such as *SNAI1, SNAI2* and *TWIST1*, may play important roles in determining the therapeutic agents that will most effectively target IBC tumor emboli for destruction

5. Acknowledgements

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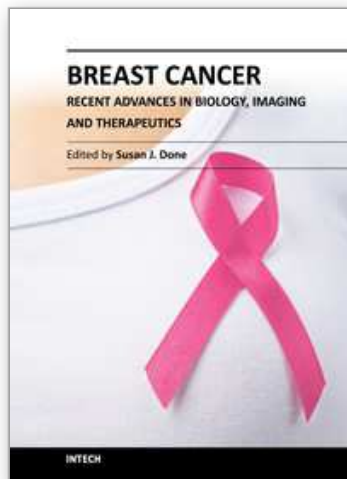
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In recent years it has become clear that breast cancer is not a single disease but rather that the term encompasses a number of molecularly distinct tumors arising from the epithelial cells of the breast. There is an urgent need to better understand these distinct subtypes and develop tailored diagnostic approaches and treatments appropriate to each. This book considers breast cancer from many novel and exciting perspectives. New insights into the basic biology of breast cancer are discussed together with high throughput approaches to molecular profiling. Innovative strategies for diagnosis and imaging are presented as well as emerging perspectives on breast cancer treatment. Each of the topics in this volume is addressed by respected experts in their fields and it is hoped that readers will be stimulated and challenged by the contents.

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