

BIM-EL localization: the key to understanding anoikis resistance in inflammatory breast cancer cells

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Cassandra L Buchheit and Zachary T Schafer*

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Department of Biological Sciences; Boler-Parseghian Center for Rare and Neglected Diseases; University of Notre Dame; Notre Dame, IN USA

Keywords: anoikis, BIM-EL, cell death, ERK/MAPK, inflammatory breast cancer, receptor tyrosine kinase

Inflammatory breast cancer (IBC) is a highly metastatic and rare type of breast cancer, accounting for 2–6% of newly diagnosed breast cancer cases each year. The highly metastatic nature of IBC cells remains poorly understood. Here we describe our recent data regarding the ability of IBC cells to overcome anoikis.

Although inflammatory breast cancer (IBC) is rare, it is highly aggressive and often affects younger patients. Compared with a 5-year survival rate for all breast cancer patients of 90%, the rate for IBC patients is only 40% and the prognosis is generally very poor.¹ IBC is characterized by the lack of a palpable primary tumor. Rather, cancerous cells flourish in the dermal lymphatic vessels and give rise to what appears to be inflammation.² Based on this unique disease progression, we hypothesized that IBC cells must be inherently resistant to anoikis, the induction of caspase-mediated cell death in response to extracellular matrix (ECM) detachment. Evasion of anoikis is necessary for metastatic progression,^{3,4} and is presumably essential for IBC cell survival in lymphatic vessels. Recently, we have uncovered a novel mechanism utilized by IBC cells to block anoikis that relies on localization of the extra long isoform of BCL2-like 11 protein (BIM-EL).⁵ Here, we discuss our findings in more detail and postulate how this information may contribute to the understanding of IBC pathogenesis and cell death.

It is well established that loss of ECM attachment in mammary epithelial cells results in a robust induction of anoikis.³ In contrast, we found that IBC cells are highly resistant to the induction of anoikis and exhibit significant anchorage-independent growth in soft agar.⁵ To

interrogate the molecular mechanism by which IBC cells survive during ECM detachment, we investigated the role of receptor tyrosine kinases (RTKs) in anoikis inhibition. Erb-b2 receptor tyrosine kinase 2 (ERBB2) and epidermal growth factor receptor (EGFR) are overexpressed (or constitutively activated) in approximately 30–50% of IBC patients,⁶ and have previously been shown to regulate intracellular signaling pathways that contribute to anoikis evasion.³ Indeed, shRNA-mediated reduction of ERBB2 or EGFR in IBC cell lines containing these respective mutations/amplifications significantly reduced the ability of IBC cells to evade anoikis and to grow in an anchorage-independent fashion.

During our efforts to ascertain the cytoplasmic signal transduction pathways responsible for anoikis evasion downstream of these RTKs, we discovered that shRNA-mediated knockdown of RTKs significantly limited activation of the mitogen activated protein kinase 1 (ERK/MAPK) pathway.⁵ To determine whether the ERK/MAPK pathway is necessary for anoikis evasion in IBC cells, we treated ECM-detached IBC cells with pharmacological inhibitors of ERK/MAPK signaling and discovered that ERK/MAPK is necessary for blockage of anoikis. In contrast, inhibition of other well-known survival pathways that operate downstream of RTKs (e.g., phosphatidylinositol-4,

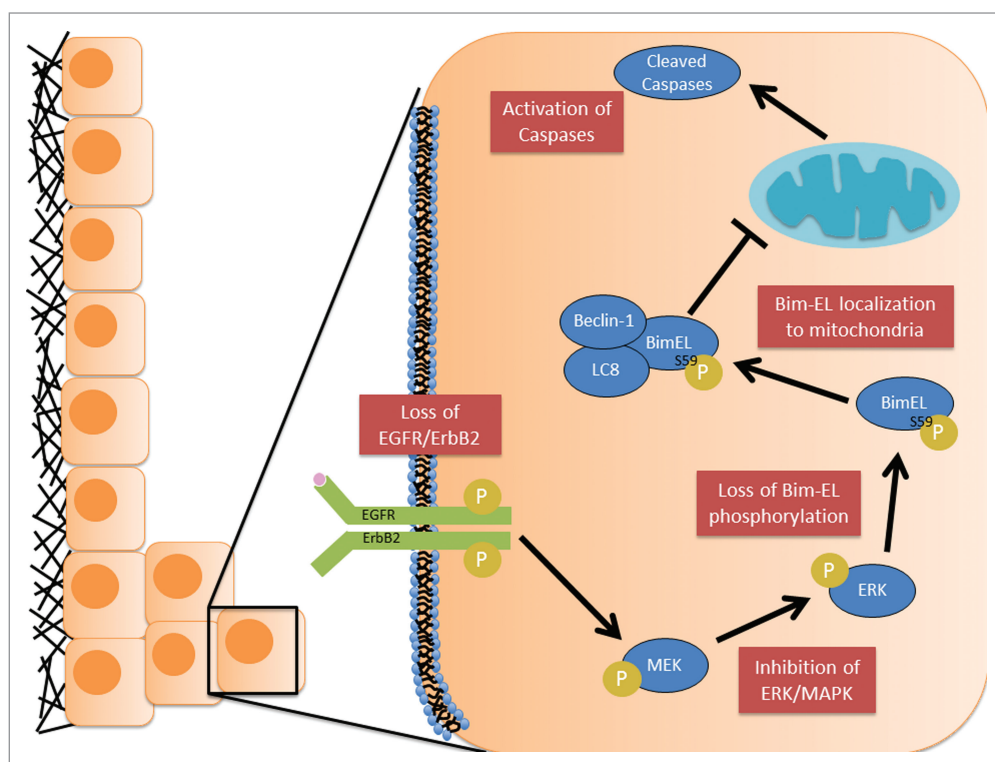
5-bisphosphate 3-kinase [PI(3)K]), did not result in specific inhibition of anoikis. Previous reports examining anoikis inhibition have implicated ERK/MAPK in the phosphorylation and subsequent turnover of the proapoptotic protein BIM-EL.^{3,7,8} To determine whether this mechanism facilitates the survival of IBC cells, we examined whether ERK/MAPK inhibition resulted in enhanced BIM-EL expression. Surprisingly, although we did observe a significant increase in BIM-EL levels when ERK/MAPK was inhibited in non-IBC breast cancer cell lines, we did not observe appreciable changes in BIM-EL levels following ERK/MAPK inhibition in IBC cell lines. Interestingly, in contrast to non-IBC breast cancer cell lines, IBC cells had high endogenous levels of BIM-EL protein. We also observed considerable BIM-EL protein in tissue specimens from IBC patients. Given the significant inhibition of anoikis observed in IBC cells, these data suggest that the activity of BIM-EL protein is antagonized in some fashion in IBC cells to block anoikis.

Interestingly, we observed a distinct electromobility shift in BIM-EL when ERK/MAPK was inhibited in IBC cells, suggesting that BIM-EL is an ERK/MAPK substrate in IBC cells.⁵ Upon further examination, we found that ERK/MAPK phosphorylates BIM-EL on serine 59. Our subsequent studies demonstrated

*Correspondence to: Zachary T Schafer: Email: zschafe1@nd.edu

Submitted: 01/19/2015; Revised: 01/19/2015; Accepted: 01/21/2015

<http://dx.doi.org/10.1080/23723556.2015.1011474>



fashion that promotes survival.¹⁰ If autophagy is not contributing to nutrient consumption in ECM-detached IBC cells, perhaps these cells utilize other mechanisms, such as fatty acid oxidation, to mitigate metabolic deficiencies. Lastly, these data identify a signaling axis that could be amenable to the design of novel chemotherapeutics and suggest that using available targeted therapies against EGFR or ERBB2 may be efficacious in IBC patients with high BIM-EL expression. Additional strategies aimed at liberating BIM-EL from the complex with BECLIN-1 and LC8 would also presumably be worth exploring to specifically eliminate ECM-detached IBC cells. We believe these novel insights into BIM-EL location in IBC cells provide an important contribution to the understanding of this unique disease and we are hopeful that they will lead to more effective chemotherapeutic approaches

that BIM-EL phosphorylation at serine 59 enables its association with the proteins BECLIN-1 and dynein, light chain, LC8-type 1 (LC8). Upon localization to this complex, BIM-EL is unable to interact with prosurvival B-cell CLL/lymphoma 2 (BCL2) family members and properly localize to the mitochondria to promote cell death. To further assess the significance of these findings, we generated the S59A mutation in BIM-EL and found that the expression of this mutant led to significantly higher levels of anoikis in IBC cells. Together, these data suggest a model by which ERK/MAPK-mediated phosphorylation of BIM-EL at serine

59 sequesters BIM-EL from the mitochondria and hence blocks anoikis in IBC cells (see **Fig. 1**).

These findings offer substantial new information on IBC pathogenesis and raise a number of important issues. First, the presence of BECLIN-1 in the BIM-EL protein complex suggests that autophagy may also be disrupted in IBC cells. Recently, the interaction of BIM-EL with BECLIN-1 was shown to inhibit autophagosome formation, supporting the possibility that IBC cells are defective in autophagy.⁹ Interestingly, autophagy has been shown to alleviate metabolic deficiencies in ECM-detached cells in a

aimed at eliminating ECM-detached IBC cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

Z.T. Schafer is supported by a Research Scholar Grant from the American Cancer Society (RSG-14-145-01) and a Career Catalyst Grant from Susan G. Komen (CCR14302768).

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