BIOGRAPHICAL SKETCH

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NAME: Andrew J. Ewald, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): aewald2

POSITION TITLE: Professor of Cell Biology, Oncology and Biomedical Engineering

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	Field of Study
Haverford College, Haverford, PA	B.S.	05/1997	Physics, Minor Biophysics
California Institute of Technology, Pasadena, CA	Ph.D.	05/2003	Biochemistry and Molecular Biophysics
UCSF, San Francisco, CA	Postdoc	07/2008	Epithelial Biology and Breast Cancer

A. Personal Statement

My lab seeks to understand how epithelial cells escape their developmental constraints and acquire the ability to spread systematically and colonize distant organs during metastasis. To accomplish this goal, we have developed imaging, genetic, and 3D “organoid” culture techniques to enable real-time analysis of cell and molecular dynamics in breast cancer. These organoid assays have been used to model normal mammary development (Dev Cell 2008; JCS 2013; Development 2014&2016, MiMB 2015, Genes & Dev. 2014, PNAS 2016a, Dev Cell 2018), test the role of the extracellular matrix in regulating breast cancer invasion (PNAS 2012; JoM 2013; Biomaterials 2013), define the minimum molecular requirements for epithelial dissemination (JCB 2014, Biology Open 2016, JCB 2018, Can Res 2000 a,b), identify the cellular phenotypes and molecular programs driving metastasis in an unbiased fashion (Cell 2013, PNAS 2016b, Nature 2019, Can Res 2020b, PLoS Comp Bio 2020), and isolate the specific contributions of the fibroblastic, vascular, and immune components of the tumor microenvironment (JCB 2020, Can Res 2020c, MCR 2000). We also extended our protocols to fresh human primary breast tumors, to metastatic site tumors from mice and humans, and to liver, esophagus, and pancreas (Nature Protocols 2020). My interdisciplinary training in physics, biophysics, and biology prepares me to collaborate across basic science, physical science, and clinical disciplines. I apply these skills in my role as Co-Director of the Cancer Invasion and Metastasis (CIM) Research Program in the SKCCC, which brings together 42 faculty from 15 departments and has both weekly seminars and an annual retreat to encourage interdisciplinary collaboration. Since 2021, I am the Director of the Department of Cell Biology (15 faculty). Our Department has historic strengths in imaging, cell migration, lipid trafficking, and cancer cell biology. The Department has an inclusive and collaborative atmosphere that facilitates the transfer of techniques and concepts between labs particularly in the areas of biosensors, imaging, and image analysis.

1. Cheung KJ, Gabrielson E, Werb Z, **Ewald AJ**, “Collective invasion in breast cancer requires a conserved basal epithelial program,” Cell. 2013 Dec 19;155(7):1639-51. PMC3941206
2. Cheung KJ, Padmanaban VP, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, **Ewald AJ**, “Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters,” PNAS, 2016 Feb 16;113(7):E854-63. PMC4763783
3. Padmanaban V, Krol I, Suhail Y, Szczerba BM, Aceto N, Bader JS, **Ewald AJ**, "E-cadherin is required for metastasis in multiple models of breast cancer," Nature, 2019 Sep;573(7774):439-444, PMC7365572.
4. Chan IS, Knútsdóttir H, Ramakrishnan G, Padmanaban V, Warrier M, Ramirez JC, Zhang H, Jaffee EM, Bader JS, **Ewald AJ**, " Cancer cells educate natural killer cells to a metastasis promoting cell state, Journal of Cell Biology, 2020 Sep 7; 219(9). PMC32645139

B. Positions and Honors

Positions and Employment

2008-2014 Assistant Professor, Johns Hopkins University, School of Medicine, Baltimore, MD
2014-2018 Associate Professor, Johns Hopkins University, School of Medicine, Baltimore, MD
2018- Professor, Johns Hopkins University, School of Medicine, Baltimore, MD
Primary: Department of Cell Biology; *Secondary:* Oncology, Biomedical Engineering
Training Grant Faculty (T32): Medical Oncology (2009-), Surgical Oncology (2011-), Medical Scientist Training Program (MSTP; 2013-), Biomedical Engineering (2014-)
2018- Founding Co-Director, Cancer Invasion and Metastasis Program SKCCC
2021- Director, Department of Cell Biology, JHU, School of Medicine, Baltimore, MD

Professional Memberships

2000- Member, American Society for Cell Biology
2001- Member, Society for Developmental Biology
2010- Member, American Association for Cancer Research
2014- Member, European Association for Cancer Research

Honors

1997 Honors in Physics: Haverford College
2011 American Association of Anatomists Morphological Sciences Award: *"For outstanding contributions to the field of epithelial morphogenesis"*
2012 American Cancer Society Research Scholar Award
2013 Keynote Speaker, NCI Physical Sciences in Oncology National Meeting, Scottsdale, AZ
2014-17 Fellow, Keith R. Porter Endowment for Cell Biology:"For exceptional contributions to cell biology"
2015 Jerome L. Greene Foundation Scholar
2015 Keynote Speaker, Gordon Research Seminar, Directed Cell Migration
2015 Society for Photo-Optical Instrumentation Engineers: Systems Biology Pioneer Award*: "For development of epithelial organoids as a platform for tissue level systems biology"*
2015 Metastatic Breast Cancer Network Research Leadership Award: "*For expansion of our basic understanding of the biology of metastasis"*
2016 Johns Hopkins University Provost's Catalyst Award
2016 Johns Hopkins University Provost's Discovery Award
2017 Theresa's Research Foundation Leadership Award
2018 Blaffer Lecturer, Department of Genetics, MD Anderson
2018 Invited Speaker, Nobel Foundation Conference on Cancer Metastasis, Stockholm, Sweden
2019 Stabler Foundation Discovery Award
2019-24 JHU Daniel Nathans Scientific Innovator Award
2020 Sackler Scholar, Mortimer and Raymond Sackler Institute for Advanced Study, Tel Aviv Univ.

Selected JHU and National Leadership Roles

2010-15 Scientific Advisory Board, IMI PREDECT: 21 site European Union wide public-private partnership to develop complex models for cancer target validation (2015 Chair).
2014-16 AACR: Steering Committee Member, Tumor Microenvironment Working Group
2014-19 Co-Director Hopkins-Allegheny Health Network Cancer Research Fund: awards $2 M/yr.
2015-6 Scientific Organizing Committee, EACR 3D Models in Cancer Research Meeting, Berlin
2015- Editor, *Journal of Cell Science*
2015-6 Scientific Program Committee: EACR Goodbye Flat Biology Conference, Berlin, Oct. 2-5, 2016
2016 Chair, Migration/Invasion Section, Tumor Biology Subcommittee, 2016 AACR Program Committee
2016-22 JHU School of Medicine Committee on Conflict of Interest (Chair 2019-22)
2016 Co-Chair of Multicellular Interactions, Tissues, and Development Minisymp, 2016 ASCB Meeting
2016 Elected Vice-Chair (2017) & Chair (2019), Gordon Research Conf. Cell Contact and Adhesion
2017 Organizer, Company of Biologists Workshop on 3D Cell Biology, Wiston House, UK, Feb
2017-8 Chair, Scientific Program Committee: EACR Goodbye Flat Biology, Berlin,
2018 CME Activity Director, The Metastatic Breast Cancer Conference, Baltimore, MD

Ad Hoc Journal Reviewer (Selected)

Biophysical Journal, Cancer Research, Current Biology, Development, Cancer Research, Developmental Cell, Journal of Cell Science, Journal of Cell Biology, MBoC, Nature, Nature Cell Biology, Nature Methods, Nature Reviews Molecular Cell Biology, PNAS, Science.

Ad Hoc Grant Reviewer (Selected)

National Cancer Institute, CDMRP Breast Cancer Research Program, NCRRR (UK), Medical Research Council (UK), Human Frontiers in Science Program, Atip-Avenir (France), Dutch Breast Cancer Foundation, Susan G. Komen Foundation.

C. Contributions to Science

1. My approach to metastasis is founded in my study of mammary branching morphogenesis. My timelapse imaging revealed that elongating ducts utilize a novel form of collective migration without ECM-directed protrusions (a). We next demonstrated that, during morphogenesis, the epithelium is transiently stratified, with low apico-basal polarity and that both result from an asymmetric cell division within the luminal epithelium (b). We recently collaborated with engineers and physicists to demonstrate that these collective groups of epithelial cells have extraordinary gradient sensitivity and to develop a novel intracellular communication theory from these data (c). Finally, we worked with civil engineers and molecular imaging specialists to decipher the molecular logic and mechanical basis of tube elongation (d).
   a. **Ewald AJ**, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Dev Cell. 2008 Apr;14(4):570-81. PMC2773823
   b. Huebner RJ, Lechler T, **Ewald AJ**, "Mammary epithelial stratification occurs through symmetry breaking vertical divisions of luminal cells," Development. 2014 Mar;141(5):1085-94. PMC3929409
   c. Ellison D, Mugler A, Brennan M, Lee SH, Huebner RJ, Shamir ER, Woo LA, Kim J, Amar P, Nemenman I*, **Ewald AJ***, Levchenko A*, "Cell-cell communication enhances the capacity of cell ensembles to sense shallow gradients during morphogenesis", 2016 Feb 9;113(6):E679-88. PMC4760786 *Co-Corresponding
   d. Neumann NM, Perrone MC, Vedlhuis JH, Zhan H, Devreotes PN, Brodland GW, **Ewald AJ**, "Coordination of receptor tyrosine kinase signaling and interfacial tension dynamics drive radial intercalation and tube elongation," Dev Cell. 2018 Apr 9;45(1):67-82. PMC5983037

2. Tumors contain diverse cancer cells with varied molecular phenotypes. We sought to understand the relative contribution of these various populations to invasion and metastasis. To do so, we cultured primary tumor organoids in collagen I gels to stimulate invasion and characterized their molecular phenotypes. We discovered that the cells leading invasion strands expressed markers of basal epithelial differentiation, such as cytokeratin-14 (K14) across diverse mouse models and patient tissue *(a)*. We then used molecular biosensors to demonstrate that luminal cancer cells can transition to a K14+ cell state to invade. Knockdown of K14 inhibited both collective invasion and distant metastasis (a,b). We next revealed that metastasis is accomplished by clusters of these migratory K14+ cancer cells that revert to a proliferative K14- state to as they form macro-metastases (b). These data led us to propose a new conceptual framework of "collective epithelial metastasis". We next used novel microfluidic devices to co-culture tumor organoids in defined proximity to a functional engineered blood vessel (c). We demonstrated that cancer cells can collectively invade into and replace the vessel wall and that these "mosaic vessels" can serve as sites of persistent intravasation. Finally, having shown that groups of cancer cells collectively invade and disseminate in clusters, we sought to understand the role of cell adhesion in metastasis. E-cadherin was viewed as a metastasis suppressor despite clear clinical data that its expression is typically preserved or enhanced in invasive ductal breast cancers (IDC) and their resulting metastases. We used genetic techniques to demonstrate that E-cadherin suppresses invasion but promotes metastasis through its role as a survival signal that limits both TGF-β and ROS signaling (d). We are currently elucidating these signaling pathways and identifying efficient therapeutic strategies to eliminate E-cadherin+ cancer cells.
   a. Cheung KJ, Gabrielson E, Werb Z, **Ewald AJ**, "Collective invasion in breast cancer requires a conserved basal epithelial program," Cell. 2013 Dec 19;155(7):1639-51. PMC3941206
   b. Cheung KJ, Padmanaban VP, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, **Ewald AJ**, "Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters," PNAS, 2016 Feb 16;113(7):E854-63. PMC4763783

c. Silvestri VL, Henriet E, Wong AD, Searson PC*, Ewald AJ*, “An engineered 3D vessel model reveals the dynamics of mosaic vessel formation and collective intravasation”, Cancer Research, 2020 Jul 14;canres.1564.2019. PMC7541732
d. Padmanaban V, Krol I, Suhail Y, Szczerba BM, Aceto N, Bader JS, **Ewald AJ**, "E-cadherin is required for metastasis in multiple models of breast cancer," 2019 Sep;573(7774):439-444, PMC7365572

3. Our data suggest that collective epithelial metastasis is a frequent mechanism in human cancer. We next sought to understand the limits of this theory and to understand the epithelial to mesenchymal transition (EMT). We started with a genetically engineered mouse with inducible Twist1 expression from the Tran Lab (a). We observed that Twist1 expression was sufficient to induce dissemination of genetically normal epithelial cells within 24-48 hours. We then demonstrated that Twist1+ cells retain epithelial gene expression and proliferative capacity during each step of dissemination (a) We next utilized this model to reveal that normal myoepithelial cells are a dynamic and efficient barrier to the dissemination of invasive luminal epithelial cells and breast cancer cells (b). Briefly, they migrate actively to enclose and restrain invasive cells and depend on both smooth muscle contractility and intercellular adhesion to do so. We then used a combination of gene expression analysis and multiplexed ELISAs to identify druggable molecular effectors downstream of Twist1. We discovered that Prkd1 is a direct transcriptional target of Twist1 and that its inhibition blocks invasion and metastasis in vivo in a murine model of basal breast cancer and both invasion and dissemination in cultures of patient tumors (c). Finally, we collaborated with the Wood lab to characterize the relative utilization of collective vs. EMT mechanisms of metastasis in human pancreatic ductal adenocarcinoma and to characterize the mutations that correlate with each mechanism (d).
   a. Shamir ER, Papallardo E, Jorgens DM, Coutinho K, Tsai WT, Aziz K, Auer M, Tran PT, Bader JS, **Ewald AJ**, “Twist1-induced dissemination preserves epithelial identity and requires E-cadherin,” The Journal of Cell Biology. 2014 Mar 3;204(5):839-56. PMC3941052
   b. Sirka OK, Shamir ER, **Ewald AJ**, "Myoepithelial cells are a dynamic barrier to epithelial dissemination," J Cell Biol. 2018 Oct 1;217(10):3368-3381. PMC6168248
   c. Georgess D, Padmanaban V, Sirka OK, Choi A, Frid G, Neumann NM, **Ewald AJ**, "Twist1-induced epithelial dissemination requires Prkd1 signaling," Cancer Research, 80 (2), 204-218 2020 Jan 15. PMC31676574.
   d. Huang W, Xia L, Jeong YJ, Chianchiano P, Serer BN, Trujilo MA, Lionheart G, Luchini G, Veronese N, Nguyen-Ngoc KV, Neumann NM, Groot VP, Singhi AD, Gaida MM, Wolfgang CL, He J, Thompson ED, Roberts NJ, **Ewald AJ**, Wood LD, "Pattern of invasion in human pancreatic cancer organoids is associated with Smad4 loss and clinical outcome," Cancer Res. 2020 May 6. PMC7335355.

4. Having focused on cancer cell-intrinsic mechanisms of metastasis, we next sought to understand how the tumor microenvironment regulated metastasis. We identified the fibrillar architecture of collagen I as a key regulator of dissemination (a), demonstrated with synthetic polymer systems that high nanoscale adhesive density is more important than the bulk rigidity of the matrix (b), and developed a novel autologous stroma co-culture assay to demonstrate that cancer associated fibroblasts induce the K14+ basal invasive molecular program in cancer cells in a NOX4 and TGFβ dependent fashion. (c). The concept that emerges is that cancer invasion and dissemination are regulated by the composition and supramolecular organization of the ECM and its attendant stromal cells. We next collaborated with Jaffee to build a novel co-culture model for natural killer (NK) cells and tumor organoids (d). We discovered that cancer cells can induce a state transition in the NK cells that causes them to lose cytotoxic ability and instead promote metastatic colonization (d). Collaborating with Bader we discovered that the state transition required DNMTs and could be prevented using blocking antibodies to either TIGIT or Klrg1 (d). Current efforts focus on reconstituting immune network function with autologous cells in both murine and human systems.
   a. Nguyen-Ngoc KV, Cheung KJ, Brenot A, Shamir ER, Gray RS, Hines WC, Yaswen P, Werb Z, **Ewald AJ**. “ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium”. PNAS. 2012 Sep 25;109(39):E2595-604. PMC3465416
   b. Beck JN, Singh A, Rothenberg AR, Elisseeff JH, **Ewald AJ**, “The independent roles of mechanical, structural and adhesion characteristics of 3D hydrogels on the regulation of cancer invasion and dissemination,” Biomaterials, 2013 Dec;34(37):9486-95. PMC3832184
   c. Hanley CJ, Henriet E, Sirka OK, Thomas GJ, Ewald AJ, " Tumor resident stromal cells promote breast cancer invasion through regulation of the basal phenotype," Molecular Cancer Research. 2020 Nov;18(11):1615-1622. PMC7642004

d. Chan IS, Knútsdóttir H, Ramakrishnan G, Padmanaban V, Warrier M, Ramirez JC, Zhang H, Jaffee EM, Bader JS, **Ewald AJ**, " Cancer cells educate natural killer cells to a metastasis promoting cell state, Journal of Cell Biology, 2020 Sep 7; 219(9) PMC32645139

Complete List of Published Work in MyBibliography: 70 total publications
Google Scholar Report 2/28/21: 12,778 total citations, h-index of 41, i10-index of 57.
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47897254/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH/NCI U01 CA217846 (MPI: J Bader, A Ewald) 09/01/2017 – 07/31/2022
Pathway Discovery and Target Validation for Outgrowth of Breast Cancer Metastases
The goal of this proposal is to utilize the heterogeneity of breast tumors as the basis for systematic unbiased analysis of the genetic, epigenetic, and gene expression drivers of metastatic spread. Aim 1: Complex trait dissection of early processes in metastasis. Aim 2: Prioritization and validation of candidate targets. Aim 3: Modulation by chemical and chemical-genetic perturbagens.

NIH/NCI U01 CA221007 (MPI: Fertig, Ewald, Tran, Popel) 04/17/2018 – 03/31/2023
Integrating bioinformatics into multiscale models for hepatocellular carcinoma
The major goal of this project is develop a novel hybrid, multiscale model that merges models of cellular signaling and molecular transport, agent-based models of growth and invasion, and compartment-based models for pharmacokinetics/pharmacodynamics (PK/PD) to bridge the molecular, cellular, and tumor scales.

NCI U54 CA210173 (MPI: D Wirtz, K Pienta) 08/26/16-07/30/21
Physical Sciences in Oncology Center Grant
The PSOC will feature three highly integrated projects: (1) the role of physical cues in collective cell invasion; (2) adhesive crosstalk in collective tumor cell invasion; and (3) the effect of oxygen gradients on sarcoma invasiveness through dynamic collagen modulation.
Role: Project 1 Investigator

Breast Cancer Research Foundation (PI: Ewald) 10/01/13 - 09/30/21
Molecular programs of breast cancer metastasis
The major goals of this grant are to: (1) define the role of epithelial and mesenchymal programs of metastasis in TNBC and (2) define the roles of tight junctions and microtubules in collective invasion.

NSF: Physics of Living Systems 1915491 (MPI: Camley, Ewald) 4/1/20-3/31/23
Collective gradient sensing and cell-to-cell variability - theory and experiment
The major goals of this grant are to: (1) test gradient sensing hypotheses in organoids with inducible differences in morphogen signaling, (2) develop data analysis methods to better quantify cellular variability, (3) understand the physical and information bounds on collective decision-making.

Completed Research Support

DoD CDMRP BCRP BC141955P1 (MPI: Inoue, Ewald) 9/30/15-9/29/18
Synthetic Biology Approach to Turn Cells Phagocytic against Breast Cancer Cells
The major goal of this grant was to develop molecular strategies to generate artificial phagocytic cells.

JKTG Foundation (MPI: Ewald, Bader, Newton, Macklin, Peyton) 1/1/17-12/31/19
Deciphering the molecular control of cancer invasion through quantitative analysis and modeling
The major goals of this grant were to develop a quantitative trait framework for analyzing specific processes in breast cancer metastasis and to integrate the resulting data into an agent based modeling framework.