BIOGRAPHICAL SKETCH

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NAME: Howlett, Niall George

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

POSITION TITLE: Professor, Cell and Molecular Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Limerick, Ireland	B.Sc.	06/1994	Biochemistry
Oxford Brookes University, U.K.	Ph.D.	01/1999	Biological and Molecular Sciences
Harvard School of Public Health, Boston	Postdoctoral	03/2001	Cancer Cell Biology
Dana-Farber Cancer Institute, Boston	Postdoctoral	10/2003	Pediatric Oncology
University of Michigan, Ann Arbor	Postdoctoral	02/2006	Human Genetics

A. Personal Statement

My laboratory studies the eukaryotic DNA damage response and the etiology of hereditary bone marrow failure and cancer susceptibility syndromes associated with defective DNA repair. We are particularly focused on the molecular pathogenesis of Fanconi anemia (FA), a rare genetic disease characterized by congenital defects, hematologic disease and bone marrow failure, myeloid malignancies and squamous cell carcinomas, and premature mortality. Therapeutic options for FA are extremely limited. To date, 22 FA genes have been identified and the protein products of these genes function cooperatively in the FA pathway to repair DNA damage and to maintain genome integrity.

A key step in the activation of the FA pathway is the monoubiquitination of the FANCD2 and FANCI proteins. Monoubiquitinated FANCD2 and FANCI localize to chromatin where they play a key role in DNA repair. Importantly, FANCD2/FANCI monoubiquitination is defective in >90% of FA patients and integral to FA patient risk for hematologic disease and bone marrow failure. My laboratory is particularly focused on gaining a greater understanding of the regulation and function of the FANCD2 and FANCI proteins in chromatin, emphasizing computational and biochemical approaches.

B. Positions and Honors

Positions and Employment

2006-2007 Research Investigator in Human Genetics, University of Michigan, Ann Arbor, MI
2007-2012 Assistant Professor in Cell and Molecular Biology, University of Rhode Island, Kingston, RI
2012-2020 Associate Professor in Cell and Molecular Biology, University of Rhode Island, Kingston, RI
2020-*present* Professor in Cell and Molecular Biology, University of Rhode Island, Kingston, RI

Other Experience and Professional Memberships

1994-2000 Member, Society for General Microbiology, UK
1998-2002 Member, American Society for Microbiology

2002-present Grant reviewer, Boehringer Ingelheim Fonds Ph.D. fellowship program, Fanconi Anemia Research Fund, Medical Research Council U.K., North West Cancer Research Fund U.K., Ohio Cancer Research Associates, Science Foundation Ireland/Health Research Board Ireland
2002-present *Ad hoc* reviewer for *Blood, Cancer Genetics and Cytogenetics, Cell Reports, DNA Repair, Human Molecular Genetics, International Journal of Biochemistry and Cell Biology, Journal of Cellular Biochemistry, Molecular and Cellular Biology, Mutation Research, Nucleic Acids Research, PLoS One, and PLoS Genetics*
2009-present Grant Reviewer: Department of Defense Bone Marrow Failure Research Program
2009-2012 Member, American Society of Hematology
2008-2012 Member, American Association for Cancer Research
2013-present Member, American Society for Biochemistry and Molecular Biology
2015-present Member, RI-INBRE Scientific Executive Committee
2016 NIH/NHLBI Program Project Grant Review panel member
2016 NIH/NCI Cancer Etiology Study Section temporary member
2017-present Member, American Society of Hematology
2019-present American Society of Hematology Scholar Award Basic/Translational Study Section

Honors

2003-2004 Lady Tata Memorial Trust International Award for Research in Leukemia (*declined*)
2003-2005 Leukemia Research Foundation Postdoctoral Fellowship Award
2008 University of Rhode Island, Division for Research and Economic Development Outstanding Contribution to Research Award
2008 Leukemia Research Foundation New Investigator Award
2011 Department of Defense Bone Marrow Failure Research Program New Investigator Award

C. Contributions to Science

1. My first significant contributions to science were as a graduate student in the laboratory of Simon V. Avery at Oxford Brookes University in the U.K. Here, we described the influence of **plasma membrane fatty acid composition on eukaryotic susceptibility to heavy metal toxicity**, using *Saccharomyces cerevisiae* as a model system. Our findings revealed for the first time that plasma membrane fatty acid composition is a major determinant of eukaryotic susceptibility to heavy metal cytotoxicity. Specifically, we discovered that an increased degree of plasma membrane fatty acid unsaturation resulted in increased lipid peroxidation and reactive oxygen species-mediated DNA damage following exposure to the heavy metals cadmium and copper, leading to increased cytotoxicity.
    a. Howlett NG, Avery SV (1997). Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of plasma membrane fatty acid unsaturation. Applied Environmental Microbiology, 63(8), 2971-2976. PMCID: PMC168595.
    b. Howlett NG, Avery SV (1997). Relationship between cadmium sensitivity and degree of plasma membrane fatty acid unsaturation in *Saccharomyces cerevisiae*. Applied Microbial Biotechnology, 48(4), 539-545.
    c. Howlett NG, Avery SV (1999). Flow cytometric investigation of heterogeneous copper-sensitivity in asynchronously grown *Saccharomyces cerevisiae*. FEMS Microbiology Letters, 176(2), 379-386.

2. During my early postdoctoral career at Harvard School of Public Health (HSPH) and the Dana-Farber Cancer Institute (DFCI), I became increasingly interested in **DNA repair** and **hereditary cancer susceptibility syndromes associated with defective DNA repair**. In the Schiestl laboratory at HSPH we developed transient fluorescence-based assay systems for the measurement of the two major mechanisms of DNA double-strand break (DSB) repair, nonhomologous DNA end joining (NHEJ) and homologous recombination (HR). We subsequently used these systems to characterize the DSB repair defect in cells from Nijmegen breakage syndrome (NBS) patients. In the D'Andrea laboratory at DFCI, we characterized the DNA repair phenotype of Fanconi anemia (FA) patient cells using these systems. Here, in related studies, we also made the seminal discovery that FA patients from the rare FA-D1 complementation group - a particularly aggressive form of FA characterized by embryonal tumors - have biallelic mutations in the *BRCA2* gene. In addition, together with our colleagues in the Look laboratory, we identified and functionally

characterized the zebrafish Fancd2 ortholog, and established that FA developmental defects occur *via* p53-dependent apoptosis.

a. Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD (2002). Biallelic Inactivation of *BRCA2* in Fanconi Anemia. Science, 297(5581), 606-609.
b. Liu TX, Howlett NG, Deng M, Langenau DM, Hsu K, Rhodes J, Kanki JP, D'Andrea AD, Look AT (2003). Knockdown of zebrafish Fancd2 causes developmental abnormalities via p53-dependent apoptosis. Developmental Cell, 5(6), 903-914.
c. Howlett NG, Scuric Z, D'Andrea AD, Schiestl RH (2006). Impaired DNA double-strand break repair in cells from Nijmegen breakage syndrome patients. DNA Repair, 5(2), 251-257.

3. In the Glover laboratory in the Department of Human Genetics at the University of Michigan, I continued my studies of the molecular basis of the DNA repair defects of FA patient cells. Here, we discovered that the **Fanconi anemia** D2 protein, FANCD2, plays a major role in the cellular response to perturbation of DNA replication, i.e. **the replication stress response**. Cells from FA-D2 (*FANCD2*$^{-/-}$) patients, as well as cells depleted of FANCD2 using siRNA, display markedly elevated levels of common chromosomal fragile site breakage. We also determined that FANCD2 associates directly with the major eukaryotic DNA polymerase processivity factor PCNA (*P*roliferating *C*ell *N*uclear *A*ntigen) *via* a central PCNA-interaction motif. These studies paved the way for recent studies that have described a central role for FANCD2 in DNA replication fork protection even under non-perturbed conditions, including new studies in collaboration with the Schildkraut laboratory at Albert Einstein College of Medicine published recently in Molecular Cell.
    a. Howlett NG, Taniguchi T, Durkin SG, D'Andrea D, Glover TW (2005). The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. Human Molecular Genetics, 14(5), 693-702.
    b. Durkin SG, Arlt MF, Howlett NG, Glover TW (2006). Depletion of CHK1, but not CHK2, induces chromosomal instability and breaks at common fragile sites. Oncogene, 25(32), 4381-4388.
    c. Howlett NG, Harney JA, Rego MA, Kolling IV FW, Glover TW (2009). Functional interaction between the Fanconi Anemia D2 protein and proliferating cell nuclear antigen (PCNA) *via* a conserved putative PCNA interaction motif. Journal of Biological Chemistry, 284(42), 28935-28942. PMCID: PMC2781439.
    d. Madireddy A, Kosiyatrakul ST, Boisvert RA, Herrera-Moyano E, Garcia-Rubio ML, Gerhardt J, Vuono EA, Owen N, Yan Z, Olson S, Aguilera A, Howlett NG, Schildkraut CL (2016) FANCD2 facilitates replication through common fragile sites. Molecular Cell, 64(2), 388-404. PMCID: PMC5683400.

4. Since starting my own laboratory at the University of Rhode Island in 2007, I have focused primarily on gaining a thorough understanding of the **function and regulation of FANCD2**, a poorly characterized *orphan* protein. A central step in the activation of the FA DNA repair pathway is the site-specific monoubiquitination of FANCD2. This step is defective in >90% of FA patients and integral to the etiology of this disease. We have made numerous important contributions to our understanding of the domain architecture and regulation of FANCD2, including the identification of a critical CUE (*C*oupling of *U*biquitin conjugation to *E*ndoplasmic reticulum degradation) ubiquitin-binding domain and bipartite nuclear localization signal, the discovery of a key role for the p21 cyclin-dependent kinase inhibitor in facilitating efficient FANCD2 monoubiquitination and DNA repair, as well as recently establishing a coordinate role for the PTEN phosphatase and the FA proteins in ICL repair.
    a. Rego MA, Harney JA, Mauro M, Shen M, Howlett NG (2012). Regulation of the activation of the Fanconi anemia pathway by the p21 cyclin-dependent kinase inhibitor. Oncogene, 31(3), 366-375. PMCID: PMC3974337.
    b. Rego MA, Kolling IV FW, Vuono EA, Mauro M, Howlett NG (2012). Regulation of the Fanconi anemia pathway by a CUE ubiquitin-binding domain in the FANCD2 protein. Blood, 120(10), 2109-2117. PMCID: PMC3437598.
    c. Boisvert RA, Rego MA, Azzinaro PA, Mauro M, Howlett NG (2013). Coordinate nuclear targeting of the FANCD2 and FANCI proteins via a FANCD2 nuclear localization signal. PLoS One, 8(11), e81387. PMCID: PMC3836817.

d. Vuono EA, Mukherjee A, Vierra DA, Adroved MM, Hodson C, Deans AJ, and Howlett NG (2016). The PTEN phosphatase functions cooperatively with the Fanconi anemia proteins in DNA crosslink repair. Scientific Reports, 6, 36439. PMCID: PMC5098254.

5. In recent years, we have become increasingly focused on gaining a greater understanding of the molecular connections between **Fanconi anemia and chromatin plasticity**. Activation of the FA pathway following exposure to DNA damaging agents is well characterized. However, we have recently discovered that the FA pathway is also potently activated upon treatment of cells with the histone methyltransferase inhibitor BRD4770, establishing that chromatin plasticity is a major determinant of the activation of the FA pathway. In collaboration with the Stewart group at the University of Birmingham, we have established that methylation of H3K4 by the SETD1A/KMT2F HMT enhances FANCD2 histone chaperone activity and protects stalled replication forks from uncontrolled degradation. Furthermore, we have recently identified a FANCD2 methyl-lysine-binding domain (MBD) with specificity for H4K20me2. Disruption of the MBD compromises FANCD2 chromatin binding and its ability to promote error-free DNA interstrand cross-link repair. Our studies describe important functional links between this human genetic disease and chromatin plasticity, and have the potential to open up a new avenue of epigenetics-based therapeutic opportunity.
    a. Paquin KL, Mamrak NE, Garzon JL, Cantres-Velez JA, Azzinaro PA, Vuono EA, Lima KE, Camberg JL, Howlett NG (2019). FANCD2 binding to H4K20me2 via a methyl-binding domain is essential for efficient DNA crosslink repair. Molecular and Cellular Biology, 39, pii: e00194. PMCID: PMC6639249.
    b. Higgs, M.R., Sato, K., Reynolds, J.J., Begum, S., Bayley, R., Goula, A., Vernet, A., Paquin, K.L., Skalnik DG, Kobayashi W, Takata M, Howlett NG, Kurumizaka H, Kimura H, Stewart GS (2018). Histone methylation by SETD1A protects nascent DNA through the nucleosome chaperone activity of FANCD2. Molecular Cell, 71, 25-41. PMCID: PMC6039718.
    c. Vierra DA, Garzon JL, Rego MA, Adroved MM, Mauro M, Howlett NG (2017). Modulation of the Fanconi anemia pathway *via* chemically induced changes in chromatin structure. Oncotarget, 8, 76443-76457. PMCID: PMC5652718.

Complete List of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/niall.howlett.1/bibliography/public/

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

R01 HL149907 Howlett (PI) 01/01/20-12/31/22
NIH/NHLBI New Directions in Hematology Research (SHINE-II) R01 Research Project Grant
Chromatin State Alterations in Fanconi Anemia Hematologic Disease and Bone Marrow Failure
The major goal of this 3-year SHINE II R01 research proposal is to elucidate the molecular underpinnings of the connections between Fanconi anemia (FA) and chromatin plasticity by, 1) determining the mechanisms of chromatin binding by the FANCD2 protein and 2) by characterizing the function of FANCD2 in chromatin remodeling.

[No grant number] Howlett (PI) 03/01/19-02/28/21
American Society of Hematology Bridge Grant
Role of the Fanconi Anemia D2 Protein in Replication Fork Maintenance
The major goals of this research proposal are to determine the roles of 1) a methyl-lysine binding motif and 2) a CDK phosphorylation cluster on the regulation of the FANCD2 protein.

T34 GM131948 Howlett and Dewsbury (M-PIs) 06/01/2019-05/31/2024
NIH/NIGMS T34 Undergraduate NRSA Institutional Research Training Grant
MARC U*STAR Training Program at the University of Rhode Island
The mission of the URI MARC U*STAR program is to develop a cadre of underrepresented students who emerge into critically-minded, discerning scientists, who are strong in fundamental research knowledge and practice, and are leaders and exemplars to their peers.

P20 GM103430 Cho (PI) 05/01/19-04/30/24
NIH-NIGMS P20 IDeA Networks of Biomedical Research Excellence Program
Rhode Island IDeA Network of Biomedical Research Excellence (RI-INBRE)
The overarching goal of the RI-INBRE program is to improve institutional research capacity for biomedical research excellence and hands-on student training in the State of Rhode Island.
Role: Program Coordinator

1828057 Craver (PI) 7/1/2018-6/30/2021
NSF Major Research Instrumentation (MRI)
Acquisition of a Confocal High Content Screening System to Enhance Bioengineering and Biomedical Research
The goal of this MRI award is to purchase and install an Opera Phenix Confocal High Content Screening System at the University of Rhode Island. This new instrumentation will allow researchers to address important questions in fields ranging from molecular biology to environmental engineering.
Role: co-PI

Completed Research Support (*partial*)
1R01HL101977-01A1 Howlett (PI) 04/01/11-03/01/17
NIH-NHLBI R01 Research Project Grant
Regulation of the monoubiquitination of the Fanconi anemia D2 protein
The major goal of this research proposal is to systematically characterize the regulation and function of the monoubiquitination of the Fanconi anemia D2 (FANCD2) protein.

BM100059 Howlett (PI) 04/01/11-04/30/14
Department of Defense Bone Marrow Failure Research Program New Investigator Award
De novo chromosome copy number variation in Fanconi anemia-associated hematopoietic defects
The major goal of this research project is to determine the role of the Fanconi anemia proteins in the prevention of both spontaneous and reactive oxygen species-induced chromosome copy number variation.

[No grant number] Howlett (PI) 04/01/11-03/30/12
Rhode Island Foundation Medical Research Grant
Role of the p21 protein in DNA double-strand break repair
The major goal of this research project is to determine the role of the p21 cyclin-dependent kinase inhibitor in the homologous recombination and nonhomologous end joining DNA double-strand break repair pathways.

1R21HL095991-01 Howlett (PI) 04/01/09-03/30/12
NIH-NHLBI R21 Exploratory/Developmental Research Grant
In Search of a New Fanconi Anemia-BRCA Pathway Gene on Chromosome 11p
The major goal of this research project is to uncover a new Fanconi anemia-BRCA pathway gene on chromosome 11p15.5 using a targeted siRNA screen and a cDNA expression library approach.

[No grant number] Howlett (PI) 07/01/08-06/30/10
Leukemia Research Foundation New Investigator Award
Regulation of the Mono-ubiquitination of the Fanconi Anemia D2 Protein
The major goals of this project are to determine the roles of two putative PCNA-interaction motifs as well as a putative CUE ubiquitin-binding domain in the mono-ubiquitination of the FANCD2 protein, and the activation of the FA pathway.