BIOGRAPHICAL SKETCH

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NAME: Olivier Loudig, Ph.D.

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

POSITION TITLE: Associate Scientist

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
Henry Poincare University, Nancy, France	B.Sc	09/91-06/95	Biology
Louis Pasteur University, Strasbourg, France	M.Sc.	09/96-08/97	Molecular & Cellular Biology
Queen's University, Ontario, Canada	Ph.D.	09/97-06/03	Biochemistry
Albert Einstein College of Medicine, NY, USA	Post-Doctoral Fellow	01/04-07/06	Epidemiology & Pathology

A. Personal Statement

I am an Associate Scientist in the Department of Biomedical Research at the Hackensack University Medical Center (HUMC), NJ, USA. My laboratory has developed biochemical methods to efficiently recover RNA and DNA, simultaneously, from formalin-fixed paraffin-embedded (FFPE) tissues, to identify genes deregulated in non-invasive and/or benign lesions (Loudig O. *et al.* 2007 and 2011, Kotorashvili A. *et al.* 2012). We have also developed and adapted gene expression assays for analysis of degraded genomic DNA, mRNAs, and microRNAs (miRNAs), using FFPE tissues, blood, plasma, and serum. Using these optimized assays, my laboratory has initiated retrospective miRNA expression analyses in non-invasive *in situ* breast lesions and identified deregulated miRNAs, which correlate with the development of invasive breast cancer. In an effort to obtain global miRNA expression data from FFPE tissues, serum, plasma and circulating exosomes, my laboratory recently optimized a barcoded cDNA library preparation protocol (Loudig O. *et al* 2017, 2018) for next-generation sequencing of minute amounts of degraded, compromised, or chemically modified small-RNAs. Using this approach to profile FFPE RNA from archived ductal breast carcinoma *in situ* (DCIS) lesions from patients with known clinical history, we further demonstrated that early miRNA expression changes correlate with the risk of subsequent invasive breast cancer development. Using both immuno-purification of tissue-specific circulating exosomes and next-generation sequencing of small RNAs, my laboratory is now developing molecular assays to detect human tumors, predict their behavior, and help clinicians diagnose relapse in cancer patients receiving therapy at HUMC.

B. Positions and honors

Positions

1998 – 2003 Teaching Assistant, Queen's University, Ontario, Canada
2003 – 2004 Research Assistant, Queen's University, Ontario, Canada
2004 – 2006 Post-Doctoral Fellow, Department of Epidemiology & Population Health and Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

2006 – 2008	Instructor, Department of Epidemiology & Population Health and Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.
2008- 2017	Assistant Professor, Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, New York
2017- Present	Associate Scientist, Department of Biomedical Research, Hackensack University Medical Center, Hackensack, New Jersey

Honors

1997	Outstanding Academic Award, Louis Pasteur University, France
1997 – 2001	Queen's University Award, Canada
2001	Ontario Graduate Award, Canada

C. Contribution to science

1- Technological development for the analysis of nucleic acids archived in paraffin embedded tissues

Tissues and biopsies archived from human patients from whom pathological data and clinical history has been recorded represent an invaluable source of information for epidemiological studies and biomarker discovery. However, the high-throughput analysis of degraded mRNA transcripts has been hindered by their small size and low abundancy. To overcome these challenges, we developed a molecular technology to restore damaged mRNA transcripts on a global scale. This methodology is based on the synthesis of short antisense DNA primers, produced from the degraded RNA, which can be used to reverse transcribe complementary sense mRNA transcripts contained in a universal "high-quality" mRNA library. With this method, short and damaged mRNA transcripts can be elongated and analyzed on cDNA microarrays to provide tissue/tumor specific gene expression data (US patent 8,497,067).

A major issue encountered when working with archived clinical specimens resides in their limited availability as well as the impossibility to obtain additional tissue material once the initial block(s) have been exhausted. To address this problem, we designed a highly efficient and robust simultaneous RNA and DNA extraction method, which provides maximum amounts of RNA and DNA, without affecting extraction yields for either of them (US patent 9,309,559). We used this method with human tissues and demonstrated its applicability and efficiency when working with older FFPE tissues. Total RNA extracted with this approach can be analyzed in single or multiplex qPCR assays, by microarrays or beadarrays, and by next-generation sequencing. Using highly degraded total RNA from FFPE specimens extracted by this method, we also designed a highly reproducible and robust cDNA library protocol for analysis of miRNA expression by next-generation sequencing. Finally, genomic DNA extracted with this method can be subjected to bisulfite treatment and analyzed for methylation patterns, and undergo next-generation sequencing. We are currently developing a next-generation sequencing method to analyze degraded FFPE RNA, but determined that the whole-genome DASL assay from Illumina provides reliable quantitative data when using archived specimens.

Loudig O, Milova E, Brandwein-Gensler M, Massimi A, Belbin TJ, Childs G, Singer RH, Rohan T, Prystowsky MB. Molecular restoration of archived transcriptional profiles by Complementary-Template Reverse-Transcription (CT-RT). *Nucleic Acids Research Methods*. 2007 35(15):e94.

Kotorashvili A, Ramnauth A, Liu C, Lin J, Ye K, Kim R, Hazan R, Rohan T, Fineberg S, Loudig O. Effective DNA/RNA Co-Extraction for Analysis of MicroRNAs, mRNAs, and Genomic DNA from Formalin-Fixed Paraffin-Embedded Specimens. *PLoS One.* 2012; 7(4):e34683.

Loudig O, Wang T, Ye K, Lin J, Wang Y, Ramnauth A, Liu C, Stark A, Chitale D, Greenlee R, Multerer D, Honda S, Daida Y, Spencer Feigelson H, Glass A, Couch FJ, Rohan T, Ben-Dov IZ. Evaluation and Adaptation of a Laboratory-Based cDNA Library Preparation Protocol for Retrospective Sequencing of Archived MicroRNAs from up to 35-Year-Old Clinical FFPE Specimen. *Int J Mol Sci.* 2017; 18(3). pii: E627.

Loudig O, Brandwein-Gensler M, Kim RS, Lin J, Isayeva T, Liu C, Segall JE, Kenny PA, Prystowsky MB. Illumina Whole-Genome DASL Platform: Assessing Its Performance in Formalin-Fixed Paraffin-Embedded Samples and Identifying Invasion Pattern-Related Genes In Oral Squamous Cell Carcinoma. *Human Pathology*. 2011; 42:1911-22.

2- Identification of early molecular markers of breast cancer to predict disease development

Breast cancer is a life-threatening disease and the most common type of cancer in the United States. Early detection is considered to be the first line of defense against this disease and has been responsible for a steep

decrease in cancer-related deaths. Breast cancer screening is routinely performed by mammography, supplemental digital breast tomosynthesis, ultrasounds, and/or MR imaging, which also allow detection of non-invasive Ductal Carcinoma *In Situ* (DCIS) lesions. DCIS lesions, which are also called stage 0 breast cancer, represent about 20% of all newly discovered breast cancers. These *in situ* lesions represent the penultimate stage before invasive disease when following a model of stepwise histological progression from atypia, to hyperplasia, to atypical hyperplasia, to carcinoma *in situ*, and ultimately to invasive disease. Women diagnosed with DCIS receive aggressive treatment even though only 5-14% of them will develop invasive disease within 10 years of the DCIS diagnosis. Although there are known clinical and pathological features as well as protein markers that can help evaluate these lesions, very little is known on the molecular pathways and markers involved in the development of invasive disease. Given the latent interval between DCIS diagnosis and subsequent invasive disease development, as well as the time required for prospectively collecting DCIS specimens, molecular analyses require the use of archived specimens.

Using an anatomically and pathologically related study model within the terminal duct lobular unit, we performed miRNA expression analyses on archived normal lobules, lobular carcinoma in situ, and invasive lobular carcinoma specimens. Our analyses revealed miRNA expression deregulation correlated with the development of invasive disease. One of the deregulated miRNAs, namely miR-375, whose expression was increased in *in situ* lesions concurrent with invasive breast cancer validated the molecular relatedness between these lesions and suggested that this miRNA may play an important role in the development of invasive disease. Using the non-tumorigenic MCF-10A cell line, which forms acini in 3-dimensional culture and provide an *in vitro* model for mammary morphogenesis, it was demonstrated that cells expressing miR-375 were unable to form acini and lost their cellular polarity. A pilot study on DCIS specimens further validated the upregulation of miR-375 expression in lesions from patients who later went on to develop invasive breast cancer. These findings suggest that early molecular events contribute to the development of invasive disease, which are detectable in archived specimens. By conducting large-scale retrospective studies, using archived breast DCIS specimens associated with banked clinical data and patient history, we are further mining for novel molecular markers associated with the development of invasive breast cancer.

Giricz O, Reynolds PA, Ramnauth A, Liu C, Wang T, Stead L, Childs G, Rohan T Shapiro N, Fineberg S, Kenny PA, Loudig O. Hsa-miR-375 is differentially expressed during lobular neoplasia and promotes loss of mammary acinar polarity. *The journal of Pathology.* 2012, 226(1):108-19.

Loudig O, Wang T, Ye K, Lin J, Wang Y, Ramnauth A, Liu C, Stark A, Chitale D, Greenlee R, Multerer D, Honda S, Daida Y, Spencer Feigelson H, Glass A, Couch FJ, Rohan T, Ben-Dov IZ. Evaluation and Adaptation of a Laboratory-Based cDNA Library Preparation Protocol for Retrospective Sequencing of Archived MicroRNAs from up to 35-Year-Old Clinical FFPE Specimen. *Int J Mol Sci.* 2017; 18(3). pii: E627

Arthur R, Wang Y, Ye K, Glass AG, Ginsberg M, Loudig O, Rohan T. Association between lifestyle, menstrual/reproductive history, and histological factors and risk of breast cancer in women biopsied for benign breast disease. *Breast Cancer Res Treat.* 2017.

3- Development of molecular tools for the detection of cancer miRNA biomarkers in serum and plasma

Exosomes have emerged as major intercellular communication vesicles, which have also been demonstrated to play critical roles in the development and progression of cancer, notably during metastasis. There is a plethora of lipid bilayer vesicles (different sizes and forms) that are released by cells in the micro-environment and the circulation, which have been demonstrated to contain proteins and nucleic acids that can have tremendous effects on the recipient cells.

We have concentrated our efforts on developing assays to purify exosomes and have been able to robustly sequence the miRNA content of circulating exosomes. Based on preliminary analyses and work that we performed with collaborators we determined that the method of purification greatly influences the data that will be obtained. Thus, we are currently concentrating our efforts on developing reproducible assays that will allow us to purify selected populations of exosomes, which will enable us to identify novel cancer-specific biomarkers. Our exosome research has been targeted toward the detection of colon, breast, lung, and prostate cancers.

Ho GY, Jung HJ, Schoen RE, Wang T, Lin J, Williams Z, Weissfeld JL, Park JY, Loudig O, Suh Y. Differential expression of circulating microRNAs according to severity of colorectal neoplasia. *Transl. Res*. 2015; pii: S1931-5244(15)71-7.

4- Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1fYMCvBL19Zke/bibliography/48323814/public/?sort=date&direction=ascending

D. Research Support
Completed Research Support

Breast Cancer Research Foundation
Period: 10/06 – 10/07
"*Gene expression profiling of benign breast tissue and risk of breast cancer*"
Role: co-Investigator
Objective: In this project the investigator is using the novel technique defined as Complementary-Template Reverse-Transcription (CT-RT) to restore the transcriptional profiles of benign breast disease archived tissues correlated with risk of breast cancer.

Breast Cancer Research Foundation
Period: 10/07 – 10/08
"*MicroRNA expression profiling of benign breast tissue and risk of breast cancer*"
Role: co-Investigator
Objective: In this project the investigator will be investigating the expression of microRNA populations in archived benign breast tissues that have been correlated with risk of breast cancer, identify and test miRNA signatures using real-time RT-PCR.

Breast Cancer Research Foundation
Period: 10/08 – 10/09
"*Gene expression profiling of benign breast tissue and risk of breast cancer: Additional groundwork to proceed to full-scale study*"
Role: co-Investigator
Objective: In this project the investigator will be using the 24,000 features DASL gene expression assay from Illumina, test its robustness on archived samples and compare results with the CT-RT method established in 2007, while using benign breast disease samples that have been correlted with risk of breast cancer.

Breast Cancer Research Foundation
Period: 10/14 – 10/15
"*Gene expression profiling of ductal carcinoma in situ (DCIS) and risk of breast cancer, and analysis of risk factors for breast cancer in two cohort studies*"
PI: Dr. Thomas Rohan
Role: co-Investigator
Objective: The goals of this project are to assess the feasibility of conducting a multi-center study of molecular changes related to risk of progression from ductal carcinoma *in situ* of the breast and risk of subsequent invasive breast cancer.

R01CA142942 (PI: Rohan)
Period: 7/1/2015 - 5/31/2016
NIH/NCI
"*MicroRNA expression in benign breast tissue and risk of Breast Cancer*"
Objective: Profile microRNAs in archived benign breast tissues with regard to the risk of breast cancer development.
Role: Co-Investigator

Current Research Support

1R01CA218429-01 (MPI: Rohan, Loudig)
Period: 9/15/2017 - 7/31/2022

NIH/NCI
“Molecular markers of risk of subsequent invasive breast cancer in women with ductal carcinoma in situ”
Objective: Profile microRNAs in archived DCIS specimens from patients who do and who do not develop invasive breast cancer to identify early molecular markers of disease progression. Additional objectives include validations of immuno-histochemistry (Cox-2, p16, ki67) and the Oncotype DX DCIS molecular assay on subsets of archived specimens.
Role: PI: 5.4 months