BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. ***DO NOT EXCEED FIVE PAGES.***

NAME: Antonis Giannakakis

eRA COMMONS USER NAME (credential, e.g., agency login): N/A

POSITION TITLE: Assistant Professor, Dept. Molecular Biology and Genetics, Health Science School, Democritus University of Thrace, Alexandroupolis, Greece.

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 Wales, School of Biosciences, U.K, supervisor: prof. J. Hemingway	B.Sc	09/2001	Human Genetics
University of Birmingham, Medical Research Center (MRC), U.K., supervisor: prof. D. Adams	M.Sc	12/2002	Immunology and Infectious diseases
Democritus University of Thrace, Health Science School, Dpt. Molecular Biology & Genetics supervisor: prof. R. Sandaltzopoulos	Ph.D	02/2008	Molecular Biology & Genetics
A-STAR Research Center, Bioinformatics Institute, Singapore, supervisor: prof. V. Kuznetsov	Post-doctoral training	08/2016	Bioinformatics & long non-coding RNAs

A. Personal Statement

I have a B.Sc. on Human Genetics at University of Wales, Cardiff, U.K. and a M.Sc. on Immunology at Birmingham University, Birmingham, U.K. I was the first Ph.D graduate at the Dpt of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece, under the supervision of Professor Raphael Sandaltzopoulos. My work was towards the development of various methodologies of differential expression (like fluorescence-based differential display) for the identification of novel oncogenes and tumor-suppressors in ovarian cancer. In parallel, I have more than 2-years of work-experience providing support as technical assistant in undergraduate laboratory courses such as “Molecular Biology I”, “Gene Expression and Signaling I” enriching his teaching skills. Along this direction, I worked for the translation of “Genes VIII, B. Lewin” for the first Greek edition (translated 12 chapters). During my Ph.D. I became a visiting scholar in Professor’s George Coukos Ph.D, MD, Medical Center at the University of Pennsylvania, Philadelphia, U.S.A. where I completed part of my Ph.D. work as well as contributed in the publication of several studies on microRNAs and cancer development under the guidance of Assistant Professor Lin Zhang M.D. I was the first to report the functional role of miR-210 in hypoxia and ovarian cancer. My great interest in Systems Biology drove me to join the Bioinformatics Institute of A-STAR, Singapore where I immediately focused my post-doctoral research on the newly discovered “dark matter” of molecular biology, the long non-coding RNAs in the context of physiological stress. I was awarded a JCO career development full research grant for the identification and functional validation of novel long non-coding RNAs as regulators of the cellular stress response to oncogenic insults. I reported the first study in mammalians, on the impact of oxidative stress in global gene expression patterns, as described in the book “Long non-coding RNAs in human diseases, K. Morris” (2016), highlighting the importance of being a non-coding RNA in physiological stress conditions (when lowering the energetic cost of induction of stress response is highly desirable). A follow-up story in collaboration with the Bioinformatics Institute of A-star, Singapore is under way on the functional significance of an oxidative stress-induced promoter-associated antisense lncRNA to oxidative metabolism and DNA damage and repair response and its bi-phasic role in physiological (normal) context *versus* breast cancer.

B. Positions and Honors

Positions and Employment

2017-2018 Senior Research Assistant, University Research Institute for Malignant Childhood Diseases**,** Choremeio Research Center of First Pediatric Unit of "Agia Sofia" Children's Hospital (Director: prof. George Chrousos), Athens, Greece.

2016-2017 Senior Research Assistant, Hellenic Institute of Pasteur, DIANA-lab (Group leader: prof. Artemis Hatzigeorgiou), Athens, Greece.

2009-2016 Post-doctoral Research, Assistant, Agency for Science, Technology and Research (A*STAR), Bioinformatics Institute, Division of Genome and Gene Expression Data Analysis (Group leader: prof. Vladmir Kuznetzov), Singapore.

2005-2007 Visiting Scholar, Center for Research on Reproduction and Women's Health, Dpt. of Obstetrics and Gynecology, University of Pennsylvania Medical Center, (Group leader: prof George Coukos), Philadelphia, USA.

Research Achievement Awards

2018 Corresponding Member of the University Research Institute for Malignant Childhood Diseases, Athens, Greece.

Professional Membership (only current membership's listed)

2019-present Hellenic Society of Biochemistry and Molecular Biology
2017-present Hellenic Association of Computational Biology and Bioinformatics

Journal/Book Editor Positions

2018-present Review Editor, *Cancer Letters*

C. Contribution to Science

1. **Identification of novel biomarkers of the tumor microenvironment and immune response in ovarian cancer.** My Ph.D was funded in 2003 by a *Heracletus* PhD fellowship by the Greek Ministry of Education regarding functional genomics and biomarker discovery in ovarian cancer. The fellowship was performed during the first two and half years at the Dpt. of Molecular Biology & Genetics, Health Science School, Democritus University of Thrace (Alexandroupolis, Greece) while the following two and half years of study were undertaken at the Medical Center of the University of Pennsylvania, PA, U.S.A. The progression of ovarian cancer critically depends on the establishment of a supportive tumor microenvironment that promotes growth and expansion of cancer cells. In my Ph.D, I designed and developed several functional genomic analysis techniques for the identification of genes up-regulated in ovarian tumors as a result of their adaptation process to two parameters of tumor microenvironment: lymphocyte infiltration and hypoxia. By establishing that tumor infiltration can be determined based on CD8A mRNA levels (a T-cell marker and a cancer prognostic indicator), I managed to identify gene expression signatures that show significant correlation with tumor infiltrating cells –not only in ovarian cancer but in 10 other cancer types– and prognoses a significant better outcome in patients with ovarian cancer. In addition, a fluorescence version of a differential display technique called ADDER, was developed to identify novel biomarkers of unknown and/or rare mRNAs whose expression is significantly correlated with T-cell enrichment in ovarian tumors and patient survival.

A. Giannakakis, A. Karapetsas, D. Dangaj, E. Lanitis, J. Tanyi, G. Coukos, and R. Sandaltzopoulos, "Overexpression of SMARCE1 is associated with CD8+ T-cell infiltration in early stage ovarian cancer," Int. J. Biochem. Cell Biol., vol. 53, pp. 389–398, Aug. 2014.

A. Karapetsas, **A. Giannakakis**, D. Dangaj, E. Lanitis, S. Kynigopoulos, M. Lambropoulou, J.L. Tanyi, A. Galanis, S. Kakolyris, G. Trypsianis, G. Coukos, R. Sandaltzopoulos, "Over- expression of *GPC6* and *TMEM132D* in early stage ovarian cancer correlates with CD8+ T- lymphocyte infiltration and increased patient survival." BioMed Research International, vol. 2015, pp. 1-9, 2015.

2. **Biochemical and molecular study of HIF1A and tumor hypoxia**. An importnat aspect of the tumor microenvironment is hypoxia which that was of great interest during my PhD work. In cancer, co-optation of

the physiological cellular response to hypoxia plays a major role in the disease progression and therapeutic efforts are directed toward the inhibition of HIF1A. This protein induces the transcription of genes that mediate the adaptive response to hypoxic stress.

A. Galanis, A. Pappa, **A. Giannakakis**, E. Lanitis, D. Dangaj, and R. Sandaltzopoulos, “Reactive oxygen species and HIF-1 signaling in cancer.,” Cancer Lett., vol. 266, no. 1, pp. 12–20, Jul. 2008.

A. Karapetsas, **A. Giannakakis**, M. Pavlaki, M. Panayiotidis, R. Sandaltzopoulos, and A. Galanis, “Biochemical and molecular analysis of the interaction between ERK2 MAP kinase and hypoxia inducible factor-1α.,” Int. J. Biochem. Cell Biol., vol. 43, no. 11, pp. 1582–1590, Nov. 2011.

3. **Genomic and transcriptomic delineation of miRNA expression dynamics in cancer and ovarian tumor microenvironment.** I went on to broaden my studies in ovarian cancer to include microRNAs (miRNAs) that may function as oncogenes and tumor- suppressors genes. I analyzed the expression profiles of miRNA genes in ovarian cancer cell lines by high-throughput RT qPCR and showed that the majority of differentially expressed miRNAs in ovarian cancer are down-regulated. These data were integrated with aCGH data from a collaborator to show, for the first time, that the general down-regulation of miRNAs observed in ovarian cancer is mainly due to frequent deletions in miRNAs loci. To confirm this observation, I analyzed ~120 ovarian cancer human specimens by miRNA microarrays. This approach identified several differentially expressed miRNAs (~30) that were independently validated in early- versus late-stage ovarian tumor specimens and confirmed that most miRNAs are down-regulated as ovarian cancer progresses. In parallel, I studied the expression profile of miRNAs in hypoxic stress response of ovarian cancer cell lines. One gene, miR-210, was significantly up-regulated under hypoxia in all cell lines. Analysis of its expression in cell lines with constitutive HIF expression yielded evidence consistent with a HIF-mediated mechanism of miR-210 induction in hypoxia. In addition, I demonstrated that the miR-210 locus is frequently deleted in ovarian cancer and that miR-210’s expression correlates significantly with gene copy number loss in human epithelial ovarian cancer. Finally, several mRNAs were identified as post-transcriptional targets of miR-210 with critical roles in cell cycle, transcription and angiogenesis.

L. Zhang, J. Huang, N. Yang, J. Greshock, M. S. Megraw, **A. Giannakakis**, S. Liang, T. L. Naylor, A. Barchetti, M. R. Ward, G. Yao, A. Medina, A. O'Brien-Jenkins, D. Katsaros, A. Hatzigeorgiou, P. A. Gimotty, B. L. Weber, and G. Coukos, “microRNAs exhibit high frequency genomic alterations in human cancer.,” Proc. Natl. Acad. Sci. U.S.A., vol. 103, no. 24, pp. 9136–9141, Jun. 2006.

A. Giannakakis, G. Coukos, A. Hatzigeorgiou, R. Sandaltzopoulos, and L. Zhang, “miRNA genetic alterations in human cancers.,” Expert Opin Biol Ther, vol. 7, no. 9, pp. 1375–1386, Sep. 2007.

L. Zhang, S. Volinia, T. Bonome, G. A. Calin, J. Greshock, N. Yang, C.-G. Liu, **A. Giannakakis**, P. Alexiou, K. Hasegawa, C. N. Johnstone, M. S. Megraw, S. Adams, H. Lassus, J. Huang, S. Kaur, S. Liang, P. Sethupathy, A. Leminen, V. A. Simossis, R. Sandaltzopoulos, Y. Naomoto, D. Katsaros, P. A. Gimotty, A. DeMichele, Q. Huang, R. Bützow, A. K. Rustgi, B. L. Weber, M. J. Birrer, A. G. Hatzigeorgiou, C. M. Croce, and G. Coukos, “Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer.,” Proc. Natl. Acad. Sci. U.S.A., vol. 105, no. 19, pp. 7004–7009, May 2008.

A. Giannakakis, R. Sandaltzopoulos, J. Greshock, S. Liang, J. Huang, K. Hasegawa, C. Li, A. O'Brien-Jenkins, D. Katsaros, B. L. Weber, C. Simon, G. Coukos, and L. Zhang, “miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer.,” Cancer Biol. Ther., vol. 7, no. 2, pp. 255–264, Feb. 2008.

4. **Characterization and functional identification of lncRNAs in response to subacute oxidative stress in normal fibroblast cells.** Early in the course of my intensive work with high throughput methodologies, I acknowledged the importance of bioinformatics science in order to facilitate multi-variant decision trees complementary to the experimental validation process. I joined A-Star’s Bioinformatics Institute in Singapore, as a post-doctoral research fellow in order to be able to formulate hypothesis at a genome-wide level and design both experimental and computational experiments that may depict better the dynamic and “emerging” (since the recent discovery of non-coding RNA transcripts) nature of gene expression. In parallel, I became greatly interested in further extending my study of ncRNAs from miRNA to long non-coding RNAs (lncRNAs) in relation to the primary cellular stress response pathway.

All living organisms sense and respond to abrupt changes in their intracellular and extracellular environment through complex signaling pathways that lead to changes in gene expression and cellular function in order to maintain homeostasis. LncRNAs comprise a large and heterogeneous group of functional RNAs that play important roles in cellular response to stressful conditions. Our understanding of the contribution of lncRNAs to the cellular stress response is still highly rudimentary. Interestingly most of the

existing reports on the role of lncRNAs in mammalian cells study the role of lncRNAs in stress adaptation, which is a secondary stress-specific response of estimating whether cells have more than or less than adequate resources to deal with that specific stressor affecting their homeostasis. The primary cellular stress response (core stress response) controls the biological assessment of the stress-inflicted cellular damages, precedes the above mentioned secondary response and is triggered in a dosage-dependent but stressor-independent manner. Oxidative stress, like H_2O_2, occurs in abrupt changes of reactive oxygen species (ROS) levels and is known as a primary stress response. In mammalian cells, oxidative stress leads *via* a complex series of phosphorylation events, to a transcriptional cascade targeting many protein-coding genes involved in regulation of the cellular redox state. However, it is well known that in response to oxidative stress, transcription is not the driving force behind the changes in protein synthesis and that the expression of a large number of genes is altered *via* post-transcriptional mechanisms including regulation of RNA stability and translation. Until recently, contribution of the non-coding transcriptome to the oxidative stress response had remained largely unexplored.

Our team from three A-star Institutes reported for the first time in mammalian cells, that transcription has a more complex role in the core stress response than previously anticipated. A systems-level RNA-sequencing analysis of human fibroblasts treated with hydrogen peroxide, demonstrated that besides a sub-set of well-known stress response mRNA genes that were upregulated in comparison to the untreated cells, the vast majority of the protein-coding genes had reduced transcript levels. In contrast, the non-coding transcriptome was strongly upregulated, together with a large number of novel transcripts. Over a thousand intergenic (distal lncRNAs, dncRNAs) and antisense RNAs arising from bidirectional promoters (promoter-associated antisense lncRNAs, paancRNAs) were detected, with the majority of stress-induced RNAs belonging to the latter group. Many of the induced novel RNAs were predominantly nuclear and did not show significant protein-coding capacity. However, among those with higher predicted protein-coding capacity, many associated with polysomes to a significant extent and surprisingly in oxidative stress conditions associated to them even more compared to mRNAs. This newly identified stress-induced lncRNAs (si-lncRNAs) comprise a genome-wide transient transcriptional stress response that precedes the changes in protein synthesis and can be generated either *via* transcriptional initiation, like the immediately-early mRNAs (paancRNAs/dncRNAs) and/or *via* a transient bi-directional pol2 promoter-pausing phenomenon that results in RNA read-through events of mRNA transcription (intronic transcripts/terminal-associated lncRNAs/small to intermediate-sized ncRNAs).

A. Giannakakis, J. Zhang, P. Jenjaroenpun, S. Nama, N. Zainolabidin, M. Y. Aau, A. A. Yarmishyn, C. Vaz, A. V. Ivshina, O. V. Grinchuk, M. Voorhoeve, L. A. Vardy, P. Sampath, V. A. Kuznetsov, I. V. Kurochkin, and E. Guccione, “Contrasting expression patterns of coding and noncoding parts of the human genome upon oxidative stress,” Sci. Rep., pp. 1–16, May 2015.

5. **Functional characterization of the role of stress-induced promoter-associated antisense lncRNAs in replication stress and breast cancer**. Transcriptional stress needs to be quickly resolved because it usually leads to clashes between the DNA and RNA synthesis machineries upon initiation of the DNA replication phase (S-phase) and/or upon DNA breaks induced by DNA damaging agents like oxidative stress (oxidative DNA damage), a phenomenon usually referred to as replication stress. Since, transcripts like paancRNAs (originating from bidirectional promoters), are the pre-dominant RNA class in oxidative stress response, we rationalized that oxidative stress-induced paancRNAs may be enriched in regulatory information that confer selective advantages to faithful DNA replication. Indeed, differential expressed levels of paancRNAs activate the DNA damage response and repair (DDR) signalling cascade, arrest cells to a G1 and/or intra-S phase cell cycle checkpoints and regulate global gene expression, not only in normal but also in breast cancer cell lines.

A. Giannakakis, G.S. Ow, O. Grinchuk, A. Ivshina, V. and I. V. Kurochkin, “Stress-induced promoter-associated antisense lncRNAs function in cellular DNA damage response,” *In preparation*, 2019.

Selected publications relevant to current application

#: Senior author; *: equal contributor

L. Zhang, J. Huang, N. Yang, J. Greshock, M. S. Megraw, **A. Giannakakis**, S. Liang, T. L. Naylor, A. Barchetti, M. R. Ward, G. Yao, A. Medina, A. O'Brien-Jenkins, D. Katsaros, A. Hatzigeorgiou, P. A. Gimotty, B. L. Weber, and G. Coukos, “microRNAs exhibit high frequency genomic alterations in human cancer.,” Proc. Natl. Acad. Sci. U.S.A., vol. 103, no. 24, pp. 9136–9141, Jun. 2006.

- **A. Giannakakis**, A. Karapetsas, D. Dangaj, E. Lanitis, J. Tanyi, G. Coukos, and R. Sandaltzopoulos, “Overexpression of SMARCE1 is associated with CD8+ T-cell infiltration in early stage ovarian cancer,” Int. J. Biochem. Cell Biol., vol. 53, pp. 389–398, Aug. 2014.
-**A. Giannakakis**, G. Coukos, A. Hatzigeorgiou, R. Sandaltzopoulos, and L. Zhang, “miRNA genetic alterations in human cancers.,” Expert Opin Biol Ther, vol. 7, no. 9, pp. 1375–1386, Sep. 2007.
-L. Zhang, S. Volinia, T. Bonome, G. A. Calin, J. Greshock, N. Yang, C.-G. Liu, **A. Giannakakis**, P. Alexiou, K. Hasegawa, C. N. Johnstone, M. S. Megraw, S. Adams, H. Lassus, J. Huang, S. Kaur, S. Liang, P. Sethupathy, A. Leminen, V. A. Simossis, R. Sandaltzopoulos, Y. Naomoto, D. Katsaros, P. A. Gimotty, A. DeMichele, Q. Huang, R. Bützow, A. K. Rustgi, B. L. Weber, M. J. Birrer, A. G. Hatzigeorgiou, C. M. Croce, and G. Coukos, “Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer.,” Proc. Natl. Acad. Sci. U.S.A., vol. 105, no. 19, pp. 7004–7009, May 2008.
-**Giannakakis A**, Zhang J, Jenjaroenpun P, Nama S, Zainolabidin N, Aau MY, Yarmishyn AA, Vaz C, Ivshina AV, Grinchuk OV, Voorhoeve M, Vardy LA, Sampath P, Kuznetsov VA#, Kurochkin IV#, Guccione E#. Contrasting expression patterns of coding and noncoding parts of the human genome upon oxidative stress. Sci Reports. 2015 May 29;5:9737.

Complete List of Published Work in Google Scholar:
https://scholar.google.com/citations?user=o-CoW3kAAAAJ&hl=en&oi=ao

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

2013-2016 Co-Principal Investigator. Identification and Functional Validation of Novel Non-Coding RNAs as Regulators of the Cellular Stress Response to Oncogenic Insults”, A-STAR, Singapore, JCO Career Development Award (CDA). Direct cost: Sing $750,000.

2003-20146 Identification of differentially expressed genes in ovarian tumors ("Heracletus"), Democritus University of Thrace (Alexandroupolis, Greece), Prinicipal Investigator: prof. Raphael Sandaltzopoulos, Greek Ministry of Education, Lifelong Learning and Religious Affairs. Direct cost: 150,000 Euros

Complete Industry and Clinical Partnership Project

N/A