CELL AND DEVELOPMENTAL BIOLOGY LABORATORY

Head of the Lab**:** Petros Marangos, Associate Professor

Lab Members

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Brief Description of the Laboratory

Our laboratory specializes in the examination of the cell cycle regulation and the DNA damage response during mammalian oocyte and early embryonic development. More specifically we determine cell cycle and DNA damage response regulators in the mouse oocyte, the pre-implantation mouse embryo and the mouse neural crest. The major directions of the research are: a) the examination of the DNA damage response in oocytes and pre-implantation embryos, b) the determination of cell cycle regulators in oocyte meiosis and the examination of their potential role in the regulation of oncogenesis, c) the cell cycle regulation and DNA damage response of mammalian neural crest stem cells.

The educational activity of the laboratory involves the teaching and logistical support of the practical exercises for the courses of Cell Biology, Developmental Biology, Reproductive Biology / Assisted Reproduction of the Department of Biological Applications and Technology.

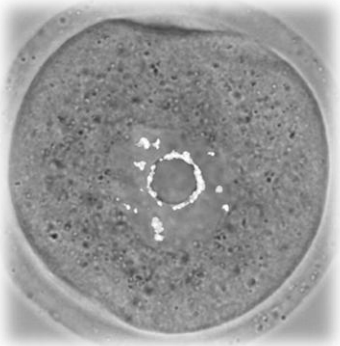
- Presentation of the Laboratory by the Ministry of Education's Network of Excellence:

http://excellence.minedu.gov.gr/

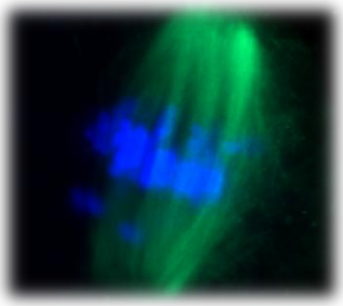
http://excellence.minedu.gov.gr/listing/465-oocytes

DNA damage response and reproductive aging

The increasing tendency of modern women to postpone childbearing has led to an increase in infertility, the development of pregnancies with chromosomal abnormalities and the pursuit for assisted reproductive treatments. Advanced maternal reproductive age has proven the main risk factor for genomic instability. A major cause of the occurrence of chromosomal abnormalities may be the inefficient response to DNA damage in oocytes. We have found that, although young oocytes lack a robust G2 DNA damage checkpoint (Marangos and Carroll, Current Biology, 2012), they establish a strong response to DNA damage in the first meiotic M-phase. This response is dependent on the activation of the spindle assembly checkpoint but also other important cell cycle regulators such as Mos/MAPK. However, aged oocytes do not show a strong response to DNA damage during meiosis I due to an inefficient spindle assembly checkpoint (Marangos *et al.*, Nature Communications, 2015). We are currently examining the DNA damage response and especially DNA repair mechanisms in young and aged mouse and human oocytes. This work will allow the identification of the effects of DNA damage on oocyte meiosis, fertilisation and embryo development and will determine potential causes of human infertility.



DNA damage in a Prophase-arrested mouse oocyte



Chromosome misalignment on an aged mouse oocyte spindle

Oocyte cell cycle regulation: Linking Meiosis to Cancer

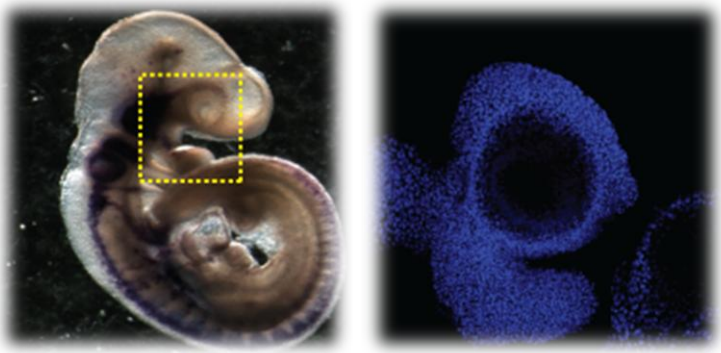
Cancer resistance to chemotherapeutic agents following prolonged exposure is a crucial problem in cancer therapy. The major reason for cancer drug resistance is the failure of damaged mitotic cells to arrest and die through apoptosis. We are examining oocyte-specific regulators as possible targets for developing new therapeutic methods to sustain drug sensitivity in cancer cells. It is known that oocyte-specific proteins, such as Mos, do not function only as meiotic regulators but are also implicated in cancer progression. In our laboratory we are examining the roles of oocyte-specific cytostatic factors in mouse oocyte M-phase, such as Mos.

Cell cycle regulation and DNA damage response in neural crest development

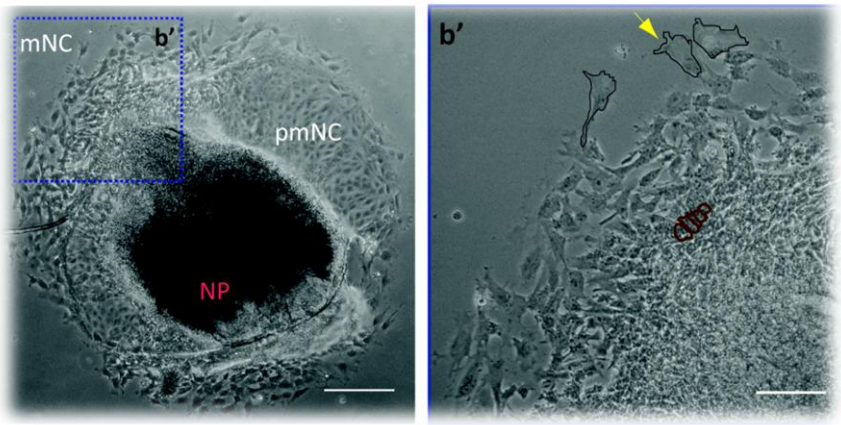
DNA damage is known to occur during embryogenesis where it promotes serious developmental abnormalities that may lead to severe disablement and even death. Moreover, some of the characteristics that cancer cells acquire are very similar to those found in stem cells involved in embryonic development. One of the most important cell populations during development is the neural crest stem cells (NCSCs). They not only act as progenitors for a vast variety of tissues but they possess the ability to migrate long distances, a characteristic also seen in metastatic cancer cells. It is known that NCSCs are responsible for serious developmental abnormalities that affect the head, the skin and the nervous, cardiac and digestive systems. The DNA damage response and cell cycle regulation of the mammalian neural crest has not yet been investigated. In our lab, we examine the regulation of the cell cycle and the DNA damage response in mammalian NCSCs. We investigate the mechanisms involved and the effects of DNA damage on the survival, migration and differentiation of NCSCs obtained from mouse embryos. We aim to determine whether DNA damage imposed on NCSCs is responsible for craniofacial abnormalities, embryonic tumors, pediatric and adult cancer and other developmental disorders. Furthermore, we investigate the cell cycle regulation during the different stages of neural crest development and specifically during migration and differentiation.

- Study of the cell cycle and development of neural crest stem cells:

https://www.jove.com/video/60051/dissection-culture-analysis-primary-cranial-neural-crest- cells-from



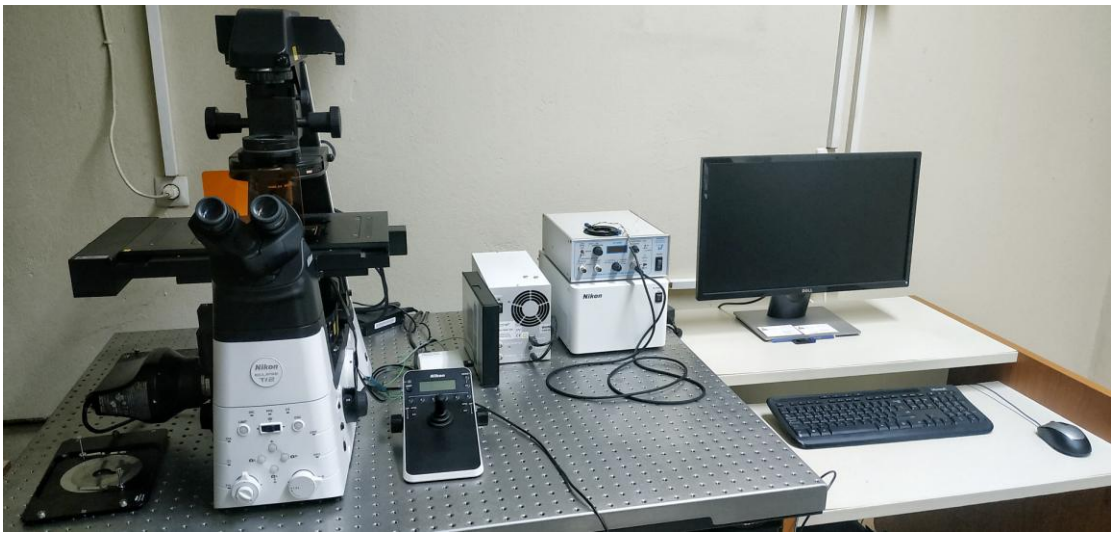
Sox10 and DNA staining in a mouse E9.5 embryo



Mouse cranial Neural Crest explant in culture
(NP:neural plate, pmNC:pre-migratory neural crest, mNC:migratory neural crest)

Laboratory infrastructure

• Wide-field fluorescence microscopy imaging system for the observation of living cells.



Fully automated Nikon Eclipse Ti2-E including LED Lumencor Sola SE II 365

• Infrastructure for the culture and micro-manipulation of oocytes and pre-implantation mammalian embryos.

Zeiss Axiovert 100TV with Narishige micro-manipulators

• Basic infrastructure for Molecular Biology and Biochemistry.

Selected publications

- Gonzalez Malagon, S. G., Dobson, L., Muñoz, A. M., Dawson, M., Barrell, W., Marangos, P., Krause, M., Liu, K. J. Dissection, Culture and Analysis of Primary Cranial Neural Crest Cells from Mouse for the Study of Neural Crest Cell Delamination and Migration. *J. Vis. Exp.* 2019, (152), e60051, doi:10.3791/60051.
- Zhang QH, Yuen WS, Adhikari D, Flegg JA, FitzHarris G, Conti M, Sicinski P, Nabti I, Marangos P, Carroll J. Cyclin A2 modulates kinetochore-microtubule attachment in meiosis II. J Cell Biol. 2017 Aug 17. 216:3133-3143.
- Nabti I, Grimes R, Sarna H, Marangos P, Carroll J. Maternal age-dependent APC/C-mediated decrease in securin causes premature sister chromatid separation in meiosis II. Nat Commun. 2017 May 18;8:15346.
- Marangos P (correspondence), Stevense M, Niaka K, Lagoudaki M, Nabti I, Jessberger R and Carroll J. DNA damage-induced metaphase I arrest is mediated by the Spindle Assembly Checkpoint and maternal age. Nature Communications. 2015. 6:8706. doi: 10.1038/ncomms9706.
- Marangos P (correspondence). Preparation of cell lysate from mouse oocytes for Western blotting analysis. Methods in Molecular Biology. 2016;1457:209-15

- Marangos P (correspondence). Preparation of cell lysate from mouse oocytes for Western blotting analysis. Methods in Molecular Biology. 2016;1457:209-15
- Ibtissem Nabti, Marangos P (correspondence), Kudo N and Carroll J. Dual-mode regulation by CDK1 and MAPK controls APC activity during meiosis I in mouse oocytes. Journal of Cell Biology. 2014 March; 204(6): 891-900.
- Carroll J and Marangos P (correspondence). The DNA damage response in mammalian oocytes. Frontiers in Genetics. 2013 June; 4:117.
- Marangos P. (correspondence) Micro-injection of Morpholino oligonucleotides for depleting Securin in mouse oocytes. Methods in Molecular Biology. 2013; 957: 153-62
- Marangos P (correspondence) and Carroll J. Oocytes progress beyond prophase in the presence of DNA damage. Current Biology, 2012 June; 22(11): 989-994.