PhD Presentation

Candidate: Stephanie Simonet

Title: The role of mitochondria and radical oxygen species in the tumoral radio-sensitization by gadolinium-based nanoparticles.

Committee: Supervisors: Dr. Dominique ARDAIL, Molecular and Cell Biology Laboratory, Lyon-Sud Medical University; Dr. Walid RACHIDI, Nucleic Acid Laboratory, CEA-Grenoble

Time: April 1st, 2016

Location: University of Lyon

Language: The presentation will be held in English

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Stephanie SIMONET
Supervisors: Dr. Dominique ARDAIL and Dr. Walid RACHIDI
Institution: University of Lyon

Introduction

Cancer is one of the leading causes of death worldwide, with head and neck cancer being ranked 6th. Head and neck cancer has a 5 years survival of less than 50%, mostly due to the high radioresistance of these tumors. Causes are multiple and current therapies available are surgery, chemotherapy, and/or radiotherapy. However, due to the high radioresistance of these tumors, as well as the local recidives and metastases, radiosensitizing strategies are being developed, one of which is nanotechnologies. Indeed, nanotechnologies open a new field of study as they are very small and therefore close to the size of biological constituents, and they are characterized as having an increased surface to volume ratio which gives them unique properties. AGuIX® are gadolinium based nanoparticles which are a good candidate as a radiosensitizer as gadolinium is a high Z atom (Z=64) and is therefore capable of producing a higher cascade of electron… In addition, AGuIX® are a good candidate for future clinical use as they are: small sized (2-5 nm), have a high colloidal stability and good Gd chelation, high EPR effect (which will increase the local dose delivered to the tumor while sparing healthy tissues), biocompatible, facilitated renal excretion, and can be used as a contrast agent for MRI which make them a theragnostic agent.

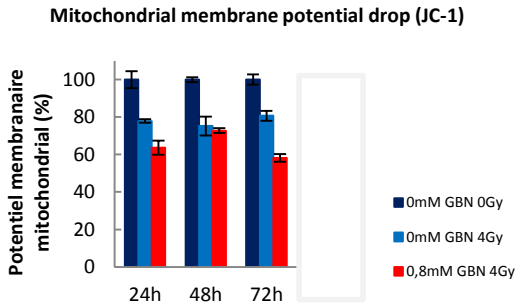
The aim of this project is to understand by which biological mechanisms radiosensitization occurs, and more specifically if mitochondria and reactive oxygen species have a decisive role in this effect.

Materials and Methods

The cellular model SQ20B, issued from a radioresistant larynx carcinoma, is used. In order to isolate the radiosensitizing conditions, ICP-AES and the surviving fraction at 4Gy was done. The conditions were validated with a clonogenic survival curve assay. The localization of AGuIX® in cells were imaged using the probes either Lysotracker (75nM 45min) or Mitotacker (200 nM 45min) and AGuIX®-Cya5.5. Mitochondrial reactive oxygen species (mROS) were marked with the Mitosox probe and quantified using flow cytometry. In order to correlate mROS produced to the drop of the mitochondrial membrane potential, this last one was measured using the JC-1 probe. Lastly, the mitochondrial DNA deletion 24h after a 4 Gy irradiation was quantified by qPCR.

Results

The radiosensitizing conditions were found to be for a treatment of a concentration 0.8 mM Gd for 24h without FBS. The amount of Gd internalized is around 0.11 pg/cell. Mitochondria have a drop in their membrane potential starting 24h after irradiation and this phenomenon remains in time (as seen in Figure below). The mitochondrial DNA deletion was observed to be increased by 6,88 times when treated with nanoparticles and 4 Gy irradiation against only 2,61 with irradiation alone.



Discussion

Prior treatment to irradiation of nanoparticle improves radiation therapy. Indeed, the EBR (10% survival) is increased by a factor of 1,3. The α is increased from 0,1593 to 0,2357 and the β factor from 0,0079 to 0,0088.

ROS are formed after combined treatment and seem to lead to mitochondrial damage, as it can be observed with the mitochondrial membrane potential drop as well as mitochondrial DNA damage increase. Confirmation remains as to the kinetics of mROS and cellular ROS.

Acknowledgements

We would like to thank the LabEx PRIMES of the University of Lyon for their financial support. Also thank-you to the lab members at the Medical School of Lyon-Sud and the CEA-Grenoble: