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Direction : Pr Bayat Sam

FIXED TERM CONTRACT
Postdoctoral position

Qualifications and education
PhD in biology, biotechnology, molecular biology

Working hours
100% over 18 months
Monday-Friday, 1 or 2 week ends per year

Salary
2900 Euros EBT

Working location
Bâtiment BioB
2280 rue de la Piscine
38400 Saint Martin d'Hères - France

CV and motivation letter to be send before the 01/05/2022
raphael.serduc@inserm.fr - serge.candeias@cea.fr

START OF THE CONTRACT 1st of June 2022

JOB DESCRIPTION

Skills

Technical

- Experience required in :
  - molecular biology (PCR / RTPCR etc ...)
  - cell biology (Culture, FACS etc ...)
  - biochemistry (WB, enzymatic assay etc ...)
  - RNA sequencing
- Desired in vivo experience (rodents)
- Knowledge and use of IT, CMMS, Excel, Windows

Human

- Autonomous and responsible
- Sense of organization
- Methodical, rigorous
- Synthesis capacity
- Compliance with regulations and procedures

Adaptability

- Flexibility
- Willingness to follow technological developments
- Responsiveness and adaptability to new situations
- Possibility of overtime in case of need for service

Relations and communication:

- Teamwork with 2 researchers, and the technical 100% on the topic / research staff of the laboratory
- Relations with the radiotherapy department CHU Grenoble

Overall working conditions:

You work within the Synchrotron Radiation and Medical Research team (Grenoble) whose goal is to develop and diffuse synchrotron X-light in the clinic. It is a multidisciplinary team made up of doctors, physicists, mathematicians and biologists. More particularly, you participate in the last stages of the clinical transfer of microbeam radiotherapy by synchrotron radiation applied to the treatment of brain tumors envisaged in the years to come.

The recruitment of the applicant will be validated by the Labex Primes COPIL (Lyon Uni) after audition.

SCIENTIFIC CONTEXT

Background

The role of the immune system in cancer progression and cancer therapy has gained increasing focus over the last decade. Macrophages are one of the major populations of immunological tumor infiltrating cells and affecting the tumor micro-environment [1]. Whereas pro-inflammatory M1 macrophages participate in anti-tumor immunity, anti-inflammatory M2 macrophages on the opposite promote tumor growth. M2 macrophages are predominant in the tumor-associated macrophage (TAM) population found at tumor beds. Modulation of TAM functional phenotype was shown to be efficient in controlling tumor growth in a murine model of glioma [2]. This

repolarization of macrophage phenotype may result from the presence of cellular debris and/or tumor antigens generated by radiation-induced glioma cell death.

We have already showed that microbeam radiation therapy (MRT), a functional method based on the spatial fractionation of microplanar synchrotron X-rays, have a better efficacy at control tumor growth and animal survival than conventional broad beam (BB) therapy in a model of rat glioblastomas [3,4]. This unique geometry significantly reduces damages to the perilesional normal tissues while delivering very high doses (hectogray) to the tumors [5]. MRT may have a higher capacity at inducing necrotic cell death and generate more/different cellular debris than BB, thereby promoting the repolarization of TAMs from M2 into M1 macrophages. We hypothesize that the differential effect already demonstrated *in vivo* is also mediated at the molecular level *in vitro*.
Thus, this project now addresses the molecular mechanisms involved in the beneficial effects of MRT on glial tumors.

This work aims at defining the effects and relevance of very high doses of radiation to tumor cells and to compare them with the effects of conventional broad beam radiotherapy (RT). We will compare *in vitro* the effects of MRT and BB on tumor cell death and characterize their secretome in both conditions. Furthermore, we will investigate whether these secreted factors are directly related to the modulation of macrophages functional phenotype after MRT. Indeed, we hypothesize that the very high doses of radiation deposited in the microbeam path promote immunogenic cell death that generates cellular debris / tumor antigens able to polarize macrophages into the anti-tumoral M1 phenotype. Our preliminary results indicate that changes in the tumor microenvironment at the vascular, cellular and molecular scales allow the shift from M2 to M1 macrophages, and these changes result in an increased therapeutic efficacy of MRT compared with BB RT. We now want to identify the underlying biological mechanisms specifically associated with MRT to explain the effectiveness of this new type of radiation therapy which appears as a novel promising tool for cancer treatment.

Methods

9L and F98 rat gliosarcoma cells will be exposed in vitro to high doses of MRT or BB radiation. We will produce dose response curves (5-25Gy) for both modalities. Radiation exposure will be perform at the ESRF facility.

We will first characterize cell death mechanisms of irradiated tumor cells exposed to BB and MRT. Tumor cell viability will be assessed using Live Cell Imaging (Izibio Celloger), flow cytometry for cell cycle changes, apoptosis (using Annexin V/ PI staining), micronuclei formation and gamma H2AX staining to differentiate between apoptosis/ necrosis / mitotic catastrophe cell death. The modulation of expression of stress and inflammatory genes in each cell line for both irradiation doses and modalities will be quantified by RT-qPCR (Cdnk1a, Ccng2, Cox2, Sod1-2, IL-6, IL-8, MCP-1, M-CSF, GM-CSF, TGFb1 …).

The secretome of tumor cells after MRT and BB irradiation will be determined using a multiplex ELISA technology to identify cytokines / growth factors specifically involved in tumor response to MRT.

In a second step, the effects of the secretome produced after tumor cell irradiation, including cytokines, growth factors, cell debris…, on macrophage phenotypes will be evaluated in conditioned media (CM) transfer experiments. Filtered (insoluble cell debris elimination) or pure media sampled after MRT or BB will be transferred to cultures of rat macrophage cell lines already available in our laboratory. After incubation in CM, macrophage phenotypes will be characterized by Live cell imaging (Izibio Celloger) assays (phagocytosis, viability, polarization assays), biochemistry tests (Arg-1 and iNOS activities) and FACS analysis. Macrophage-induced cell death with CM from irradiated tumor cells will also be assessed using conventional assays.

[1] Mantovani A, Germano G, Marchesi F, Locatelli M, Biswas SK. Cancer-promoting tumor-associated macrophages: new vistas and open questions. Eur J Immunol 2011;41:2522–5. doi:10.1002/eji.201141894.
[2] Weigert A, Johann AM, von Knethen A, Schmidt H, Geisslinger G, Brüne B. Apoptotic cells promote macrophage survival by releasing the antiapoptotic mediator sphingosine-1-phosphate. Blood 2006;108:1635–42. doi:10.1182/blood-2006-04-014852.
[3] Bouchet A, Potez M, Coquery N, Rome C, Lemasson B, Bräuer-Krisch E, et al. Permeability of Brain Tumor Vessels Induced by Uniform or Spatially Microfractionated Synchrotron Radiation Therapies. Int J Radiat Oncol Biol Phys 2017;98. doi:10.1016/j.ijrobp.2017.03.025.
[4] Bouchet A, Brauer-Krisch E, Prezado Y, El Atif M, Rogalev L, Clec'h C Le, et al. Better Efficacy of Synchrotron Spatially Microfractionated Radiation Therapy Than Uniform Radiation Therapy on Glioma. Int J Radiat Oncol Biol Phys 2016;95:1485–94. doi:10.1016/j.ijrobp.2016.03.040.
[5] Eling L, Bouchet A, Ocadiz A, Adam J-F, Kershmiri S, Elleaume H, et al. Unexpected Benefits of Multiport Synchrotron Microbeam Radiation Therapy for Brain Tumors. Cancers (Basel) 2021;13:936. doi:10.3390/cancers13050936.