Cellular Hypoxia Imaging with Phosphorescent Sensors

Alyssa Balva*[1], Andrey Klymchenko[2], Martin Sophie[3], Marlene Deschuyter[4], and Rémi Pelletier[5]

[1]Faculté de Chimie, Université de Strasbourg – université de Strasbourg – France
[2]Équipe MPB, Laboratoire de Bioimagerie et Pathologies, Faculté de Pharmacie, UMR 7021 – université de Strasbourg, Centre National de la Recherche Scientifique – France
[3]Équipe ONKO3T, Laboratoire de Bioimagerie et Pathologies, Faculté de Pharmacie, UMR 7021 – université de Strasbourg, Centre National de la Recherche Scientifique – France
[4]Co dernier, Équipe ONKO3T, Laboratoire de Bioimagerie et Pathologies, Faculté de Pharmacie, UMR 7021 – université de Strasbourg, Centre National de la Recherche Scientifique – France
[5]Équipe MPB, Laboratoire de Bioimagerie et Pathologies, Faculté de Pharmacie, UMR 7021 – université de Strasbourg, Centre National de la Recherche Scientifique – France

Résumé

Brain tumors are currently the most frequently diagnosed type of cancer in children and young adults. These tumors are characterized by a hypoxic heart, where oxygen levels can go to below 0.5%, while normal brain oxygen levels (physioxia) are around 5% O2. There is an emerging need to develop 3D in vitro models to reproduce the full complexity of the initial tumor. In actual state of the art, the few studies using patient-derived tumoroids do not study the oxygen level heterogeneity which is a crucial point to reproduce as faithfully as possible a patient- derived model.
To validate the presence of hypoxic heart in our *in vitro*, 3D tumoroid models derived from brain tumor, we aimed to use oxygen sensitive nanosensors developed in our lab. To this end, tumoroids are cultured in a rigid matrix mixed with nanosensors, to detect oxygen levels by direct fluorescence microscopy imaging. For this purpose, we use two types of luminescent oxygen-sensitive nanosensors based on polymeric nanomaterials (nanorods and nanoparticles). They contain energy-donating fluorescent dye (cyanine or rhodamine) combined with O2-sensitive acceptors phosphorescent dye (platinum porphyrin), to obtain two colors ratiometric luminescent systems involving a Förster resonance energy transfer (FRET) to amplify the oxygen sensitivity while minimizing the impact on oxygen levels. Nanoparticles are formulated by nanoprecipitation and nanorods are obtained by sonicating nanofibers produced by electrospinning.

First, we optimized the formulation of the nanoparticles by variying the loading in donor and acceptor. This led us to determine an optimal acceptor/donor ratio of 0.01 for two pairs of dyes, with dye loading up to 50wt% with respect to the polymer. Based on these results, we produced the corresponding nanorods with the same compositions. The resulting nanoparticles are, ranging in diameter from 30 to 50 nm, and nanorods obtained have a typical of 400-600 nm and a length around 5 μm. Then, we introduced our nanosensors in

*Intervenant

the matrix. Imaging of different tumoroid showed that the nanoparticles localized inside the tumoroid and penetrated the cells. However, nanorods, which have a larger structure than nanoparticles at around 100 nm, do not tend to penetrate tumoroids, but accumulate all around them, regardless of tumoroid size. Moreover, nanomaterials do not inhibit cell proliferation, enabling them to be validated as biological tools for use in living models.
Nanoparticles compared to nanorods seem more promising for studying hypoxia in these 3D models. Varying the amount of oxygen in the cultures is necessary to determine if it is possible to recreate an oxygen gradient in these in vitro brain tumor models. Further observations are planned, notably by testing different percentages of matrix to vary its rigidity.

Mots-Clés: Tumoroid, Hypoxia, Phosphorescence, Nanomaterials, FRET