DESCRIPTION FOR THE GENERAL PUBLIC

Modeling phosphorylation by human thymidine kinase 1. A crucial step in the rational design of thymidine-analogue radiosensitizers.

Radiotherapy, often the inevitable cancer treatment method, despite being used and improved very extensively for decades, produces very dangerous side effects arising from ionizing radiation action on healthy tissues. This situation is even worsened in case of hypoxic solid tumor cells, which are up to three times more radioresistant than the oxygenated ones, and therefore require much higher therapeutic doses of radiation. One of the methods employed to modulate this effect is the use of so-called radiosensitizers in combined radio-chemotherapy, for instance 5-substituted uracil derivatives (5-XU), which are believed to both selectively sensitize tumor cells and work with the hypoxic ones.

From this group, 5-iodo and 5-bromo-2'-deoxyuridine (5-IU and 5-BrU) have been extensively studied and even put on clinical trials. 5-XU incorporated into DNA undergoes dissociation after attaching the electron from water radiolysis, forming a reactive nucleobase radical that is prone to induce damage in the labeled DNA. This mechanism allowed us to computationally propose new, even more promising radiosensitizers in terms of their dissociative electron attachment (DEA) behavior.

However, efficient DEA is only a necessary condition for a potent radiosensitizer. In order to work 5-XU must be also incorporated into DNA. Otherwise, it may happen that new compound has an expected DEA profile but does not incorporate into the cellular DNA, therefore, remaining useless. The very first step of DNA sensitization *in vivo* is the phosphorylation of 5-XU. The first phosphate is added by human thymidine kinase 1 (hTK1), second and third - by thymidylate kinase and nucleoside diphosphate kinase. The final product, nucleoside triphosphate can be incorporated by DNA polymerases. It is required for every new radiosensitizer to be a good kinases and polymerases substrate. Because of exceptional substrate specificity of hTK1, one can safely assume that if 5-XU undergoes phosphorylation by hTK1, it will also work with the remaining enzymes - the first step of phosphorylation is thought to be the bottleneck for the whole process and is the topic of current study.

Thus, the project objectives are: to build a reliable hTK1 model that would be used to check whether the proposed 5-XU are good substrates for the enzyme, to calculate the phosphorylation reaction kinetic/thermodynamic characteristic and to decide whether synthesis and laboratory experiments on these 5-XU are worth of trying. The study will consist of three main parts, all conducted with the computational chemistry methods. In the first part the hTK1 model will be constructed from available experimental data and subjected to molecular dynamics simulations. After that, the mechanism of action of the enzyme will be identified with the hybrid quantum mechanics/molecular mechanics methods. Finally some of the proposed radiosensitizers, promising for their DEA profile, will be tested against their potential to be phosphorylated.

This approach will allow us not only to better understand the important biological reaction which mechanistic details are still unknown, but also to use this approach to scrutinize a set of possible radiosensitizers against their ability to be a hTK1 substrate. Such concept should allow to carry out time-consuming and expensive syntheses and cellular studies only for those nucleosides which turned out to be good hTK1 substrates.