Development of dual MGMT-proteasome inhibitors

Irina Zhirkina

University of Basel, St. Johanns-Ring 19, Basel, Switzerland
irina.zhirkina@unibas.ch

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that rescues cells from guanine O6-alkylation. This mechanism protects cells from malignant transformation, but in already existing tumours it decreases the efficacy of chemotherapy based on DNA-alkylating agents [1]. Another more widely used cancer therapy is based on proteasome inhibition. The proteasome is a sophisticated protein complex that plays a central role in protein degradation in eukaryotic cells. Since cancer cells strongly rely on high protein turnover, proteasomal degradation becomes vital for tumours [2].

In this work we aimed for the development of bispecific molecules that can do both: inhibit proteasome and cause MGMT degradation. Two series of molecules which comprise O6-benzyl guanine attached to the proteasome targeting scaffold were created. O6-Benzylguanine should covalently modify C145 of MGMT, thus causing its degradation, and the proteasome targeting part should covalently bind to the beta-5 subunit of the proteasome 20S core particle. First, compound activity was measured with western blotting. Then we developed a fluorescent cell model that allowed us to assess proteasome inhibition in a more productive way with flow cytometry. Furthermore, we investigated if the proteasome inhibition caused by some of our molecules was MGMT-dependent. For this, molecules were tested in a cell line expressing mutated C145A MGMT that should not be modified by benzylguanine moiety. Bispecific molecules appeared to inhibit proteasome in an MGMT-independent manner. Hence, we found molecules that function as dual proteasome-MGMT inhibitors, even though the two mechanisms are performed independently within the cell.

[1] P. Bai, T. Fan, G. Sun, et al., *DNA Repair*, **2023**, 123, 103449.
[2] E. Manasanch, R. Orlowski, *Nature Reviews Clinical Oncology*, **2017**, 14(7), 417–433.