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THE REGULATION OF PROTEOSTASIS IN CANCER

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RESTORING IMPAIRED FUNCTIONS OF MUTANT TUMOR SUPPRESSOR P53 USING SYNTHETIC MODULATORS

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Introduction. In ~50% of tumor cases inactivation of p53, a key oncosuppressor and transcription factor, is caused by mutations that primarily affect DNA-binding domain. Oncogenic missense mutation Y220C is the ninth most common for p53 and is annually observed in ~ 100,000 new diagnosed cancer cases worldwide. Presence of this mutation disturbs tertiary structure of the p53 DNA-binding domain that further leads to destabilization of the whole protein, its partial denaturation and loss of transcriptional activity. Selective small molecule modulators of p53(Y220C) mutant can be used to structurally stabilize the protein and restore its impaired transcriptional functions.

Materials and methods. We investigated the interaction of 2nd generation derivatives of previously reported compound MB725 with recombinantly expressed and chromatographically purified p53 and p53-Y220C proteins. For that we used two biophysical methods – surface plasmon resonance to measure interaction Kd and differential scanning fluorimetry to estimate protein thermal stability in presence of the compounds. After that we used colorimetric MTS assay to evaluate cytotoxicity of the compounds using in cells with varying p53 status (wild type, knocknout, Y220C mutant).

Results and conclusions. The compounds demonstrated a several fold increase in affinity to recombinant p53-Y220C compared to the previously described analogs, however induced thermal stability of p53-Y220C mutant increased modestly. We found that HUH7(p53-Y220C) and MCF7(p53-Y220C) were more sensitive to the treatment than MCF7(p53wt) and MCF7(p53-/-).

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SMALL MOLECULE MODULATORS OF MUTANT P53 DEMONSTRATE SELECTIVE CYTOTOXICITY TOWARD P53-Y220C CELL LINES

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Introduction. Transcription factor p53 plays a pivotal role in several critical cellular processes, including cell-cycle regulation, DNA repair and apoptosis. Missense-mutation Y220C is the ninth most frequent for p53 and is recorded globally in about 100,000 new cases annually of malignant tumors. Y220C mutation reduces thermal stability of the protein that causes disturbance of its tertiary structure and partial unfolding. A promising strategy of stabilizing the mutant p53 is based on using specific small molecule modulators that bind to narrow hydrophobic cavities formed as a result of mutation-induced changes in protein tertiary structure.

Materials and methods. We explored how 2nd generation derivatives of previously reported MB725 could restore biological activity of p53-Y220C mutant. As one of the biological models we use breast carcinoma MCF7(p53wt) and its two genetically modified variants, p53-/- and p53-Y220C, and also hepatocellular carcinoma HUH7(p53-Y220C). MCF7(p53-/-) was generated using CRISPR/Cas9 knockout. MCF7(p53-Y220C) was generated using lentiviral transduction of p53-Y220C into MCF7(p53-/-). Cytotoxicity of the compounds was assessed using colorimetric MTS assay. Cell proliferation and viability was monitored in real time using xCELLigence real time biosensor analysis.

Results and conclusions. The compounds demonstrated substantial selectivity towards HUH7(p53-Y220C) and MCF7(p53-Y220C) cell lines compared to MCF7(p53wt) and MCF7(p53-/-).

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