Sensitization of pancreatic and colorectal cancer to radiotherapy, chemotherapy and inhibition of PD-1 expression by newly developed proprotein convertase inhibitors

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Abstract

Background

The pancreatic ductal adenocarcinoma (PDAC) has a bad prognosis with delayed diagnosis that significantly impedes the use of therapeutic resection. Similarly, colorectal cancer (CRC) is often diagnosed at an advanced stage. In advanced situations, palliative chemotherapy has modest activity, and immune checkpoint inhibitors or radiotherapy failed to improve survival. The proprotein convertases (PCs) are enzymes involved in the biological activation/maturartion of various protein precursors crucial for the malignant phenotype of PDAC and CRC tumors, and resistance to various anticancer treatments.

Methods

We generated a collection of small molecules using computer-aided virtual screening and identified small molecules and repurpose approved drugs with inhibitory activity against Furin, the only convertase with published crystallographic structure. 15 molecules with significant inhibitory activity were identified and tested in syngeneic mice with PDAC and/or CRC tumors or on organoids alone or concomitant with chemotherapy (Gemcitabine or 5-Fluorouracil), and radiotherapy. PD-1 expression was analyzed in activated T cells treated with these Furin inhibitors.

Results

We identified 15 molecules with significant virtual inhibitory activity against Furin with computerized screening. Among these, 5 generated molecules (I0, I1, I10, I13, I19), significantly reduced tumor progression and enhanced survival and sensitivity to radiotherapy in syngeneic mice. Using human cancer organoids in the presence of some of these molecules, the latter were more sensitive to radiotherapy and chemotherapy. Treatment of activated T cells with these Furin inhibitors repressed significantly PD-1 expression.

Conclusion

These findings suggest the potential efficacy of Furin inhibitors in the sensitization of PDAC and CRC to treatment, and the potential immune sensitization through inhibition of T cells exhaustion, that may contribute to the development of efficient therapeutic strategies for patients with these cancers.

Results

Figure 1: (Schematic representation of the immune escape mechanism involving PD-1 expression by T cells and their exhaustion, (2) the conversion of protein precursors by the convertases (PCs) and their implication in PD-1 expression and CTL function (Cancer Res. 2019 Oct 1;79(19):5008-5021). (3) identification and selection of PCs inhibitors (I0 -I9 -I10 -I11 -I13) by VLS and drug repositioning strategies based on the catalytic site of the convertase Furin and in vitro enzymatic assay.

Figure 2: Furin expression in pancreatic and colon cancers. Furin expression pattern is altered in pancreatic and colon cancer tissues while compared to normal tissues derived from the same patients.

Figure 3: Effect of PC inhibition (PDX or PDX2) on cancer cells sensitization to radiotherapy and chemotherapy using spheres and mice bearing tumor xenografts. PCT: PCs inhibitor, PDX: PCs inhibitor, IR: Irradiation.

Figure 4: Furin inhibitors repress spheroids proliferation. Following incubation of CT-26 Spheroids with the indicated identified furin inhibitors their growth was observed and measured after 24, 48 and 72 hours.

Figure 5: Furin inhibitors decrease spheroids invasion. After treatment by the indicated furin inhibitors, spheroids were cultured on collagen at 1mg/ml, and observed at 48 hours.

Figure 6: Furin inhibitors sensibilization.

Figure 7: Inhibition of Furin activity represses PD-1 expression in T cells. Flow cytometry analysis of PD-1 expression in T cell activated by CD3 in the absence or presence of indicated identified furin inhibitors.

Conclusions

These and other findings highlight the potential use of PC inhibitors to increase the anti-tumoral immune response and could act as novel immunotherapeutic approach in cancer used alone or as adjunct therapy.

References


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