Contribution ID: **11** Type: **not specified**

p21: a new player in colon cancer cell radioresistance.

Radiation therapy is the most effective cytotoxic therapy to treat localized solid cancers. With the introduction of charged particle radiotherapy (such as proton therapy), the area of irradiated healthy tissue surrounding the tumor was further decreased. Unfortunately, radiotherapy resistance remains a major obstacle in tumor eradication, thus making it crucial to disentangle the complex molecular mechanisms underlying tumor resistance.

The cyclin-dependent kinase inhibitor p21, encoded by the CDKN1A gene, is a tumor suppressor critical for reducing cellular proliferation in response to different stress signals, including DNA damage. Interestingly, it has been demonstrated also an oncogenic role of this protein, due to its anti-apoptotic and pro-survival functions.

With the aim of elucidating the chain of events that takes place in the cell upon irradiation and better evaluate the role of the p21 pathway in response to both X-rays and proton therapy treatments, the molecular mechanisms activated upon X-rays and proton beam irradiation were studied.

As a model, we used the HCT116 colon cancer cell line and its derivative HCT116 p21KO generated using the CRISPR-Cas9 gene editing technique.

Results from our laboratory performed on these two isogenic colon cancer-derived cell lines suggest a role for p21 in radioresistance. In fact, HCT116 p21KO cells were more sensitive to both X-rays and proton beam irradiation in comparison with their parental counterpart. Moreover, to better mimic the shrinkage effect of radiation therapy on solid cancers, 3D spheroids were also used. HCT116 parental, and p21KO cells spontaneously formed spheroids in ultra-low attachment plates. Notably, while parental spheroids showed a reduction in diameter 10 days after X-rays and proton irradiation but still maintained a proper 3D organization, the p21KO spheroids completely disaggregated. Furthermore, the viability of the p21KO spheroids drastically dropped in response to X-rays and proton irradiation, and the analysis of PARP cleavage and activation of Caspase 3 highlighted an increase in apoptosis, particularly in p21 null cells.

Taken collectively, these data suggest that the absence of p53-dependent responses through p21 enhances the sensitivity to irradiation. This study revealed a dichotomy in p21 role: in addition to its canonical tumor-suppressive role, it seems to hold a radioprotective function in these cancer cells that, when depleted for p21, are considerably more prone to apoptosis. These findings could set the stage for future studies based on therapies targeting p21 in combination with charged-particles radiotherapy.

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