

**Stroma irradiation promotes tumor invasion and metastasis by suppressing angiogenesis**

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Radiotherapy is successfully used to treat a large variety of cancers. However, recurrence after radiotherapy is associated with increased local invasion, metastatic spreading and poor prognosis. While it is generally assumed that the increased aggressiveness of relapsing tumors is due to the selection of tumor cells resistant to radiation-induced apoptosis, other mechanisms may be involved. Recently it was reported that the anti-tumor effect of radiotherapy involves induction of endothelial cell death and disruption of angiogenic tumor-associated vessels. To address the question whether the tumor stroma may be involved in promoting the increased aggressiveness of tumors relapsing after radiotherapy, we established a model in which we characterized the long-term effects of radiotherapy on angiogenesis and analyzed their consequences on tumor growth, invasion and metastasis.

We report here that ionizing radiation of the prospective tumor stroma results in a sustained impairment of growth factor-driven (i.e. Matrigel plug assay) and tumor angiogenesis. Tumors growing within a previously irradiated stroma have reduced growth while they display increased hypoxia, necrosis, local invasion and distant (lung) metastasis formation. Tumor cells recovered from tumors grown within an irradiated stroma retain a stable invasive phenotype under normoxic conditions in vitro and an increased metastatic capacity in vivo. Importantly, cells with increased in vitro invasiveness and in vivo metastatic capacity are also obtained in vitro through repeated cycles of culture under hypoxic (0.1% pO\(_2\)) and normoxic (21% pO\(_2\)) conditions. Gene expression profiling and functional experiments demonstrate that the Cyr61-αVβ5 pathway is critically involved in this process. In particular the αVβ3/αVβ5 integrin inhibitor EMD121974 (Cilengitide) fully suppresses enhanced in vitro invasion and prevents lung metastasis formation of tumors growing within an irradiated microenvironment.

Based on these results we propose that radiotherapy promotes tumor invasion and metastasis of relapsing tumors through the sustained impairment of angiogenesis and subsequent hypoxia-driven selection of aggressive tumor cells. Our data point to a critical role of the tumor stroma in promoting the aggressive progression of tumor recurrences after radiotherapy and identify αVβ5/αVβ3 integrins as potential therapeutic targets to improve outcome in patients with post-radiation recurrences. More generally, these results anticipate possible long-term side effects of anti-angiogenic drugs in cancer therapies.