IDENTIFICATION OF BIOMARKERS AS INNOVATIVE TOOLS FOR PREDICTION OF ABSCOPAL EFFECTS INDUCED BY HYPOFRACTIONATED RADIOTHERAPY

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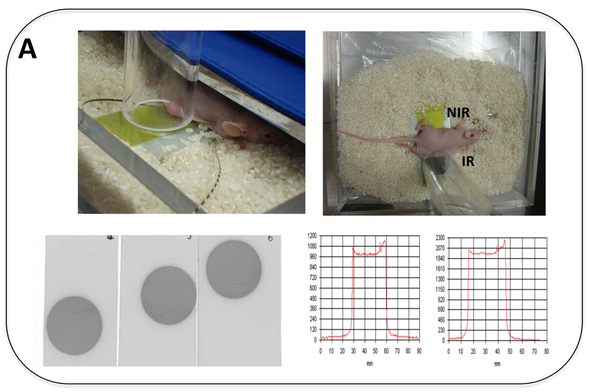
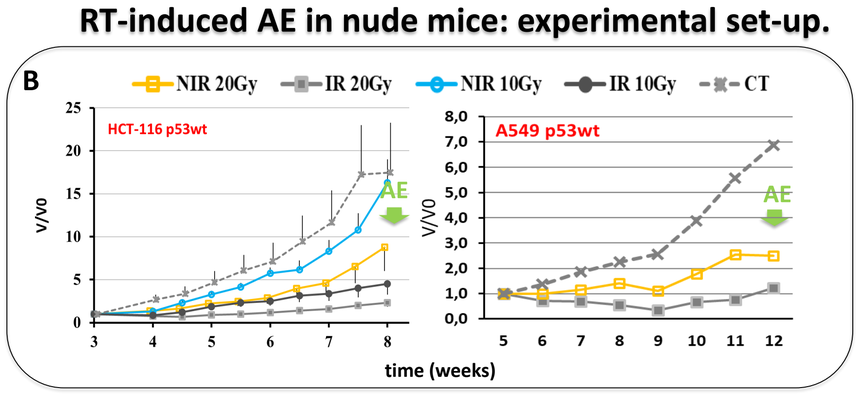
BACKGROUND AND RATIONALE

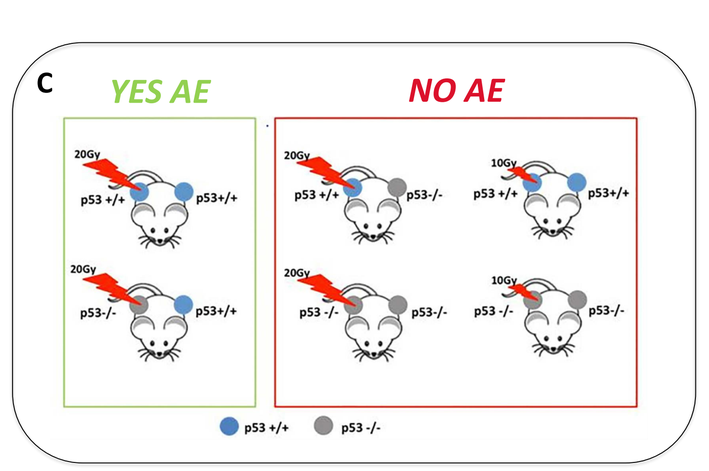
The abscopal effect (AE) or bystander effect following radiotherapy (RT) has been observed in preclinical models and in sporadic clinical cases. At present albeit the immune system has presumably roles in AE, the molecular mechanisms involved have not been clarified yet. We demonstrate AE in colorectal (HCT116) and non-small cell lung cancer (A549) xenografts tumors in nude mice. High dose rate (20 Gy) and p53 wild type (wt) occurrence are required to trigger AE in studied pre-clinical models, constituting an ideal tool to dissect molecular actors involved in AE. Accordingly, our studies are currently shedding lights on one of the molecular mechanisms involved, which could contribute in the planning of novel RT treatments for better sterilizing non-irradiated microscopic disease and / or any micro-metastasis in cancer patients scoring higher loco-regional and distant disease control and higher overall survival.

RESULTS

RT-induced AE in nude mice: experimental set-up.

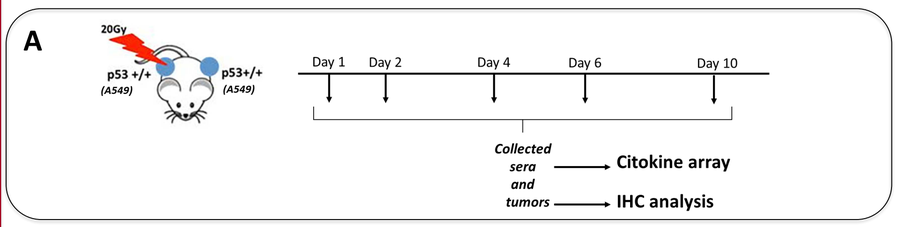


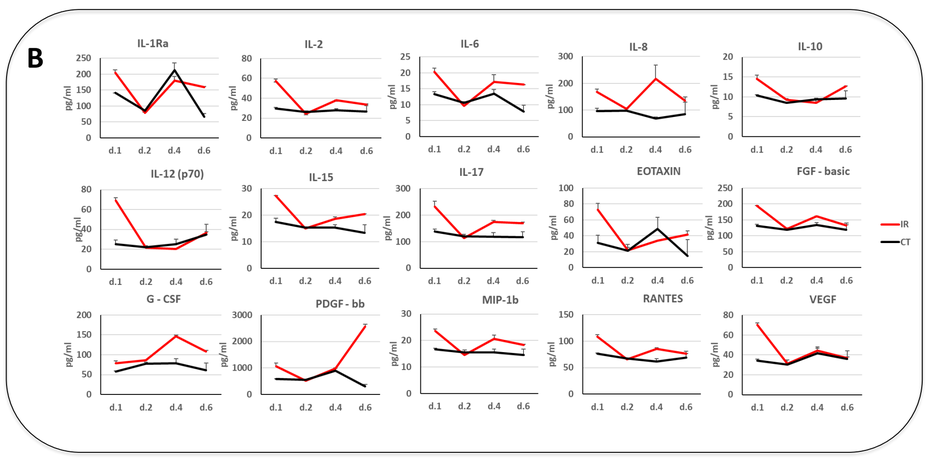


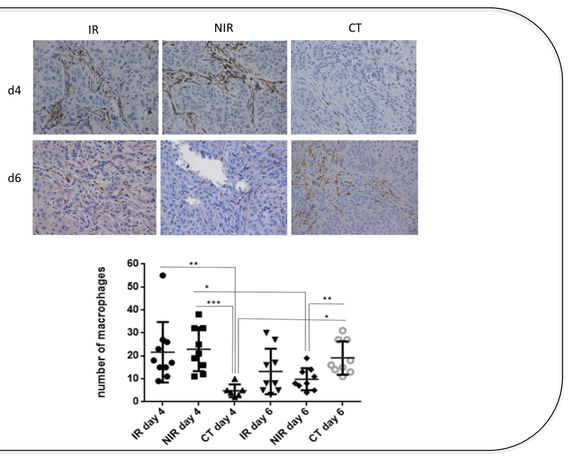


Previous studies allowed the identification of an experimental model in nude mice to study RT induced AE (A). Human cancer lines (HCT116, A549) implanted laterally and contralaterally, after tumor establishment, one lesion / animal was RT treated (IR) leaving well-shielded the whole mouse including contralateral lesion (NIR). Tumor growth was thereafter followed (B). Results showed that high dose rate (20 Gy) and p53wt occurrence in NIR tumors are required to trigger AE in our experimental models.

Exploring secretory factors involved in AE

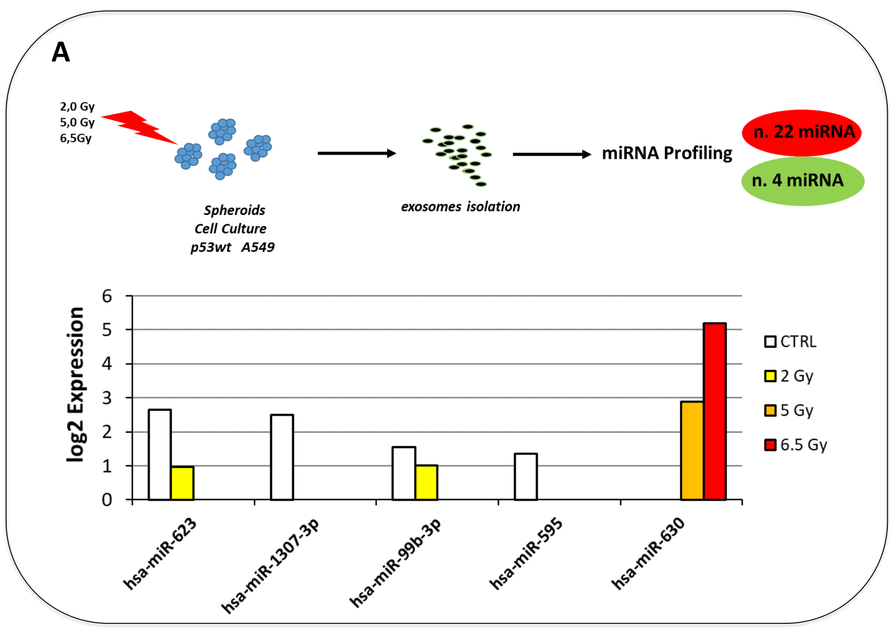


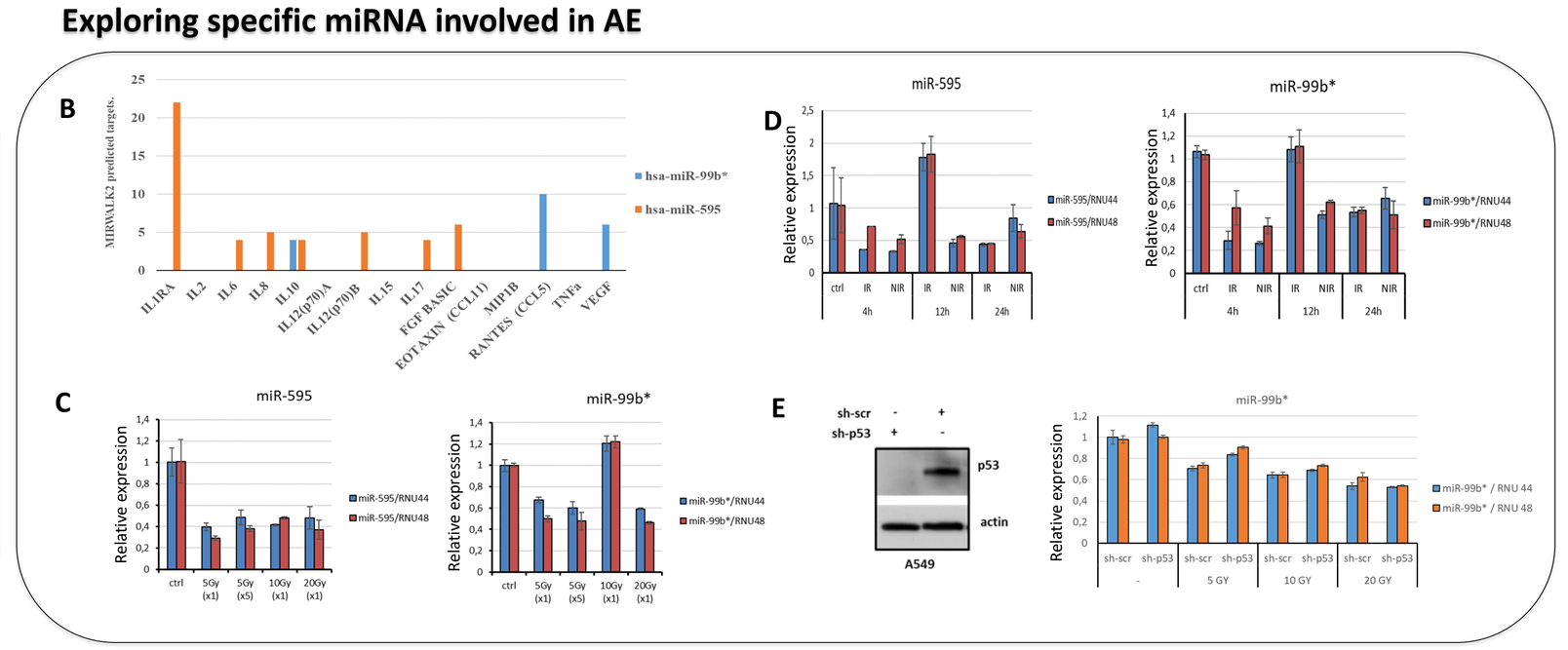




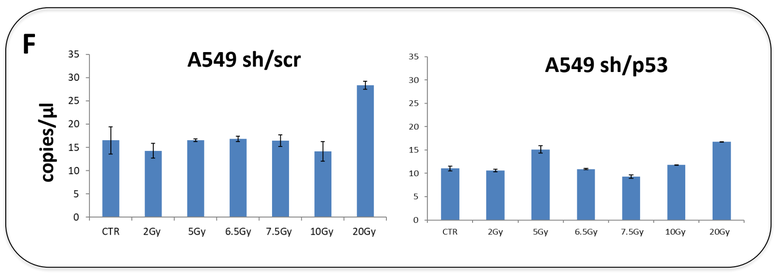
To identify secretory factors and host cells potentially involved in AE samples were collected as described in A, and sera analyzed with Bio-Plex kit detecting either murine (23-Plex) and Human Cytokine (27-Plex). Results (B) revealed upon RT significant modulations oh human cytokines mostly at 24hrs post-RT. Tumors, collected as reported in (A), were analyzed by histo- and immuno-chemistry, staining for F4/80 (macrophage), Ly6G (monocytes, granulocytes and neutrophils) and NKp46 (NK). Results (C) revealed that at day 4 post-RT a significant higher infiltration of macrophages was scored in both IR and NIR tumors compared to CT.

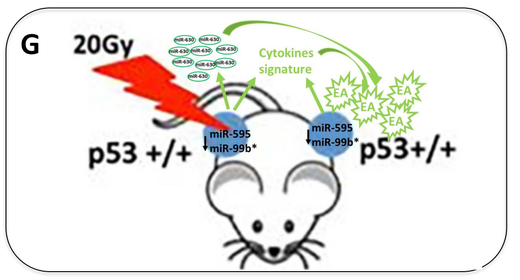
Exploring specific miRNA involved in AE





To explore specific miRNA involvement in AE, experimental plan (A) identified miRNA modulated in the exosome isolated by A549 spheroid culture upon RT. Of interest, the identified cytokine signature constitute predicted target of downmodulated miRNAs (miR-595, miR-99b*) suggesting potential regulatory network (B). The RT induced downmodulation of identified was validated in vitro (C) and in vivo (D), revealing similar effects not only in directly (IR) tumor but also in contralateral not-irradiated (NIR) (D), which occur in a wtp53 independent fashion (E). Among RT upregulated miRNAs, we focused on miR-630, because reported as oncosuppressor in lung cancer. Noteworthy, the RT induces miR-630 upregulation occurred in a wtp53 dependent manner, since completely abrogated in exosomes from sh/p53 A549 cells (F). Moreover, miR-630 upregulation was revealed in IR but not in NIR contralateral tumors (data not shown). Overall, achieved results suggest novel potential molecular mechanisms involved in RT-induced AE (G).





CONCLUSIONS

AE has been observed in both preclinical models and in sporadic clinical cases following RT. We demonstrate that RT high dose rate (20 Gy) and wtp53 occurrence are required to trigger AE in colorectal (HCT116) and non-small cell lung cancer (A549) NIR xenograft tumors in nude mice. Albeit most of the AE studies are reported in full immunocompetent mice, we sustain that the characterized nude mice AE model might constitute an ideal tool to identify molecular actors involved in AE. Accordingly, albeit RT induced AE acts through several mechanisms, our studies are currently shedding lights on one of the molecular mechanisms involved, which could contribute in the planning of novel RT treatments for better sterilizing non-irradiated microscopic disease and/or any micro-metastasis in cancer patients scoring higher loco-regional and distant disease control and increasing overall survival.

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