EphA4 receptor activation mechanism in colorectal cancer cells in the radiotherapy context

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INTRODUCTION

Colorectal cancer (CRC) is the second most common malignancy diagnosed in women and third in men, being the fourth most common cause of cancer mortality worldwide (Jemal et al., 2011). Radiotherapy (RT) is widely used as a neoadjuvant therapy for patients with advanced rectal cancers. However, tumor recurrence tends to be more aggressive with invasive metastatic conditions and shorter survival expectancy after preoperative RT (Vicini et al., 2003; Sauer et al., 2004). In previous studies, we demonstrated that the progeny from HT-29 CRC cell line submitted to 5 Gy irradiation (F1 5Gy) displayed an EMT-like phenotype. These cells exhibit an increase of EphA4 activation with ERK1/2 as a downstream pathway of this receptor. Together, PI3K and EphA4 coordinate cell migration and invasion in this model (Fig. 1) (Bastos et al., 2014; Marcondes et al., 2016). These cellular events could be responsible for the high rates of therapeutic failure promoting local invasion and metastasis in rectal cancer after RT.

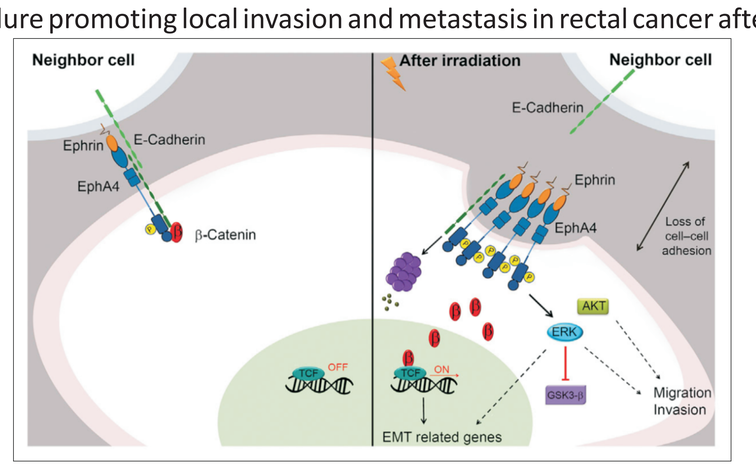
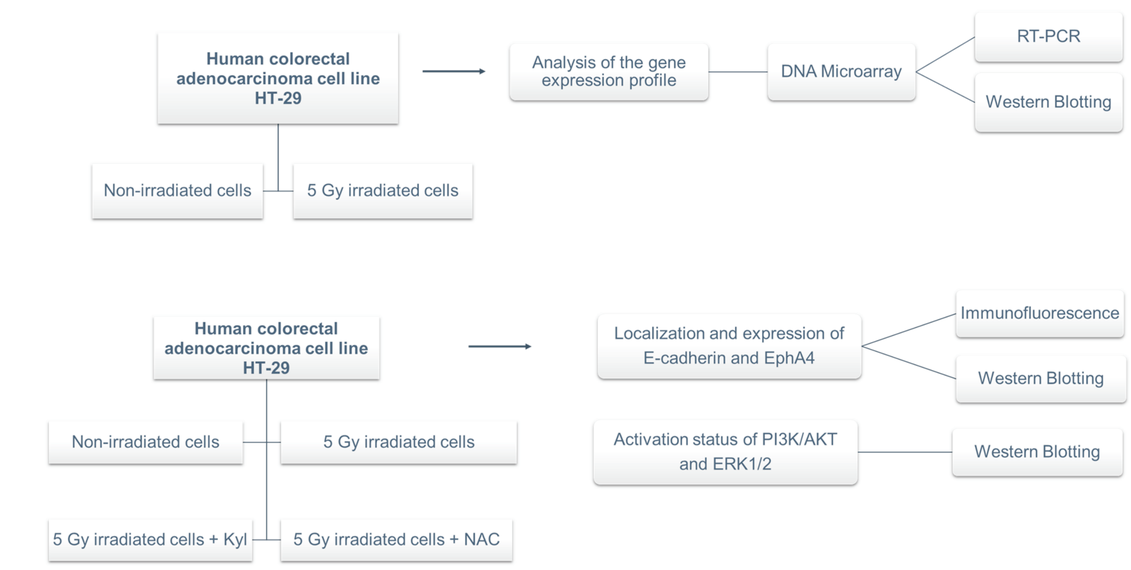


Fig. 1. Illustration of the signalling pathways induced in the progeny of colorectal cancer. Unirradiated (left) and irradiated (right) cells (Marcondes et al., 2016

OBJECTIVE

Analyze the mechanism involved in the activation of EphA4 in the radiotherapeutic context, as well as to evaluate the use of signaling pathway inhibitors as a therapeutic strategy for rectal cancers.

METHODS



PRELIMINARY RESULTS

Table 1. Microarray analysis of the main genes that are predicted to EMT process and the Ephrin genes bind to the EphA4

Gene Symbol	Gene Assignment	Fold-Change (IR vs. Control)
SNAI2	**Snail homolog 2 (Drosophila)**	2,25
STAT3	Signal Transducer and Activator of Transcription 3	1,39
EFNA4	**Ephrin-A4**	1,35
SIP1	Survival of Motor Neuron Protein Interacting Protein 1	1,31
VIM	Vimentin	1,22
SNAI1	Snail Homolog 1 (Drosophila)	1,17
TGFB1	Transforming Growth Factor Beta 1	1,17
CDH1	E-cadherin (epithelial)	1,15
EFNB1	Ephrin-B1	1,14
EFNA2	Ephrin-A2	1,10
EFNA5	Ephrin-A5	1,09
CDH2	N-cadherin (neuronal)	1,05
TGFBR1	Transforming Growth Factor Beta Receptor 1	1,04
EFNB2	Ephrin-B2	1,00
EPHA4	**EPH receptor A4**	-1,06
ZEB1	Zinc Finger E-box Binding Homeobox 1	-1,07
ZEB2	Zinc Finger E-box Binding Homeobox 2	-1,08
TWIST1	Twist Homolog 1 (Drosophila)	-1,24

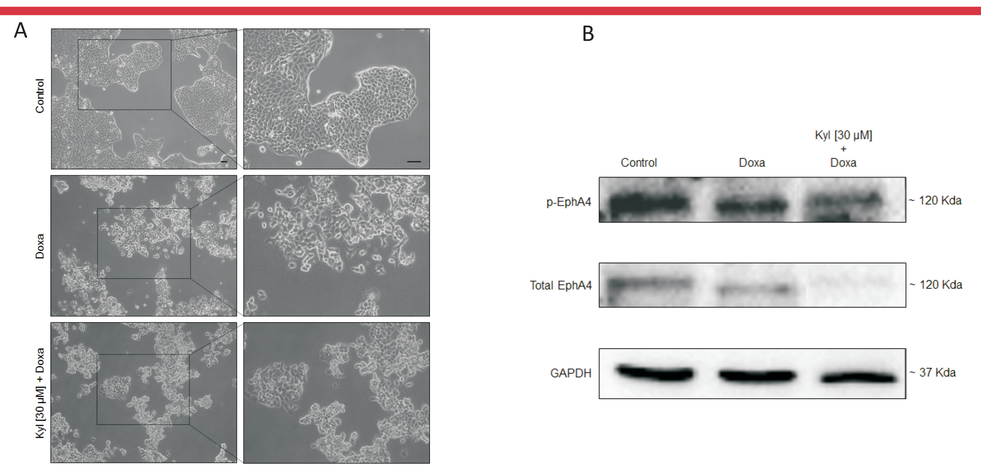


Fig. 2. HT-29 cells were pre-teated for 1 h with kyl (30 µM) and after with doxasozin (23 µM) the cells were maintained for 24 h. (A) Cell morphology analyzed using phase-contrast microscopy. Scale bar 50 µm. (B) Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels. GAPDH protein was used as a loading control.

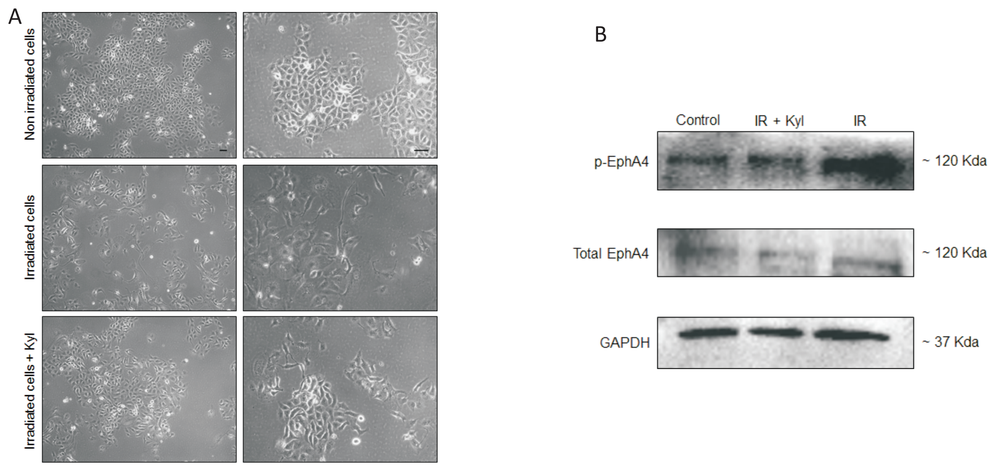


Fig. 3. HT-29 F1 cells, the group Irradiated + kyl were pre-treated for 1 h with 30 µM of Kyl and submited to irradiation. (A) Cells morphology of the HT-29 F1 cells analyzed using phase-contrast microscopy. Scale bar 50 µm. (B) Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels. GAPDH protein was used as a loading control.

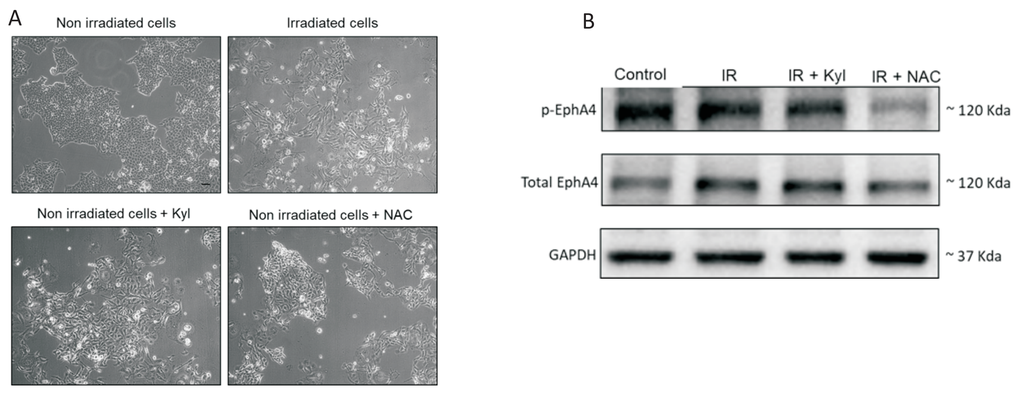


Fig. 4. HT-29 F1 cells, the group Irradiated + kyl were pre-treated for 1 h with 30 µM of Kyl and the group Irradiated + N-acetyl cysteine (NAC) were pre-treated for 1 hr with 5mM of NAC after the cells were submited to irradiation. (A) Cells morphology of the HT-29 F1 cells analyzed using phase-contrast microscopy. Scale bar 50 µm. (B) Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels. GAPDH protein was used as a loading control.

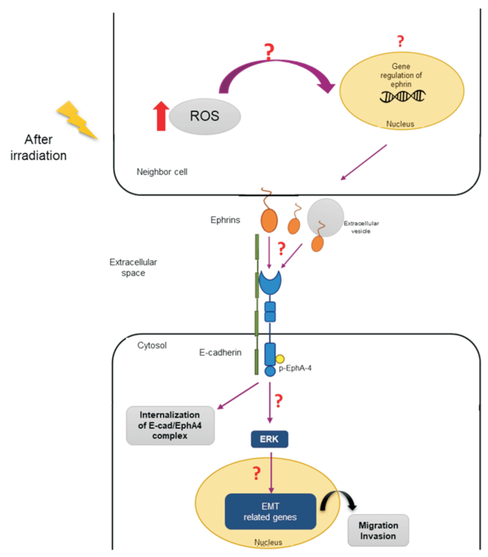


Fig. 5. Illustration of the signaling pathways induced in the progeny of colorectal cancer and the possible activation mechanism of EphA4. EphA4 receptor phosphorylation may occur by binding to its ephrin ligands. Once activated, EphA4 leads to downstream signaling, such as ERK1/2, and the internalization of the E-cadherin/EphA4 complex, which culminates in migration and invasion. The Ephrin/EphA4 binding can occur in the cell-cell contact or the ephrins can be secreted into the extracellular space. When this occurs, ephrins can be released from the cell surface in a soluble form or into extracellular vesicles and activate Eph receptors without cell-cell contact. Finally, the increase in the expression of ephrins may occur by a mechanism triggered free radicals, generated by the irradiation.

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