EphA4 receptor as a potential therapeutic target in colorectal cancer

Josiane Weber Tessmann[1], Murilo Ramos Rocha[1], Renata Binato[2], Eliana Abdelhay[3], José Andrés Morgado-Díaz[1]

[1]Programa de Oncobiologia Celular e Molecular, INCA, Rio de Janeiro, Brasil [2]Laboratório de Célula Tronco, INCA, Rio de Janeiro, Brasil, [3]Centro de Transplante de Medula Óssea (CEMO), INCA, Rio de Janeiro, Brasil.

INTRODUÇÃO

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 treatment for advanced rectal tumors in order to reduce tumor size. This approach reduces the risk of local recurrence in comparison with surgery alone. However, this confers only a small impact on distant metastasis formation (Glynne-Jones et al., 2013). 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 derived from HT-29 CRC cell line submitted to 5 Gy irradiation displayed an EMT-like phenotype characterized by downregulation of E-cadherin and increased migratory and invasive potentials (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.

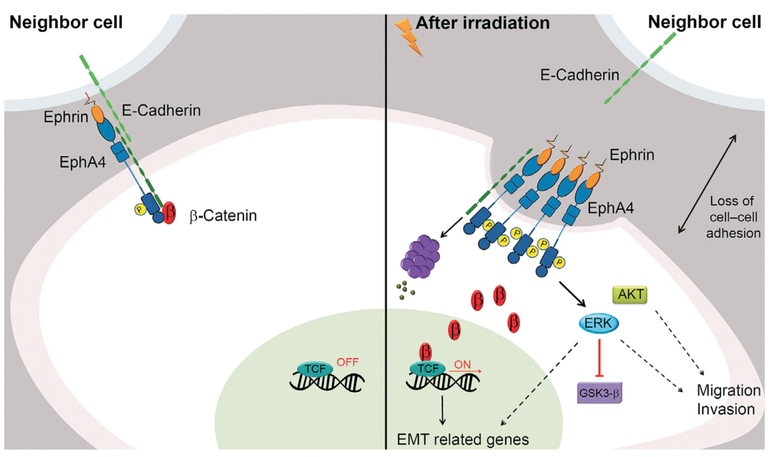
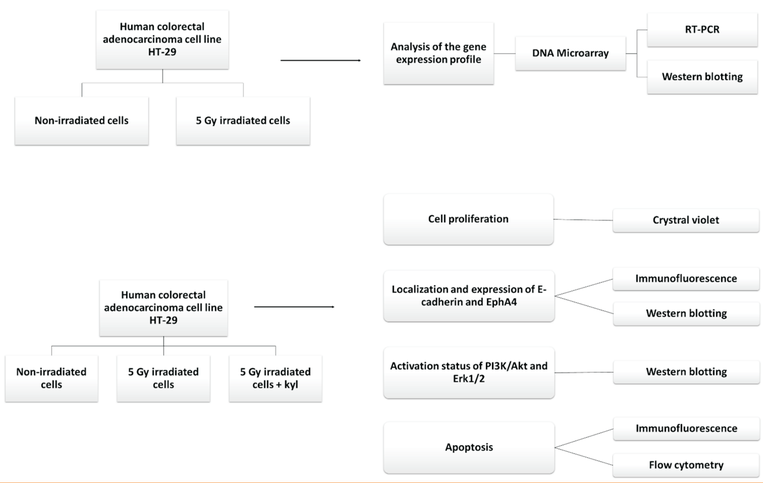


Fig. 1. Illustration of the signalling pathways induced in the progeny of colorectal cancer. Unirradiated (left) and irradiated (right) cells (Marcondes et al., 2016).

OBJECTIVE

Analyze the use of EphA4 as a therapeutic target for CRC. For this, we will use a small peptide designated KYL that competitively inhibit Ephrin binding to EphA4 receptor.

METHODS



PRELIMINARY RESULTS

Table 1. Microarray analysis of the main genes involved in the EMT process and the Ephrin genes that bind to the EphA4 receptor.

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

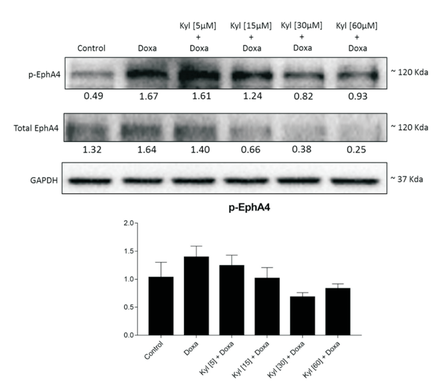
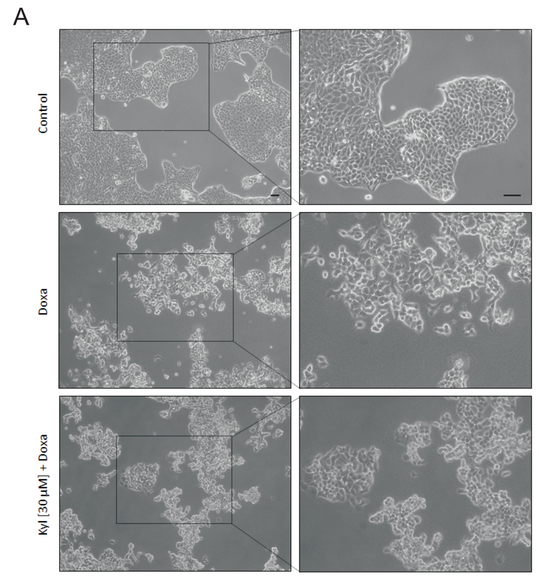


Fig. 2. Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels in lysates derived from HT-29. The cells were pretreated for 1 hr with different concentrations of Kyl (5, 15, 30 and 60 µM) and after the cells were treated with doxazosin (23 µM) for 15 min. GAPDH protein was used as a loading control. Numbers below the figure represent the ratio of band's optical density as fold change of protein expression normalized by GAPDH. Data presented as the mean ± SEM of three independents experiments. Doxa: doxazosin



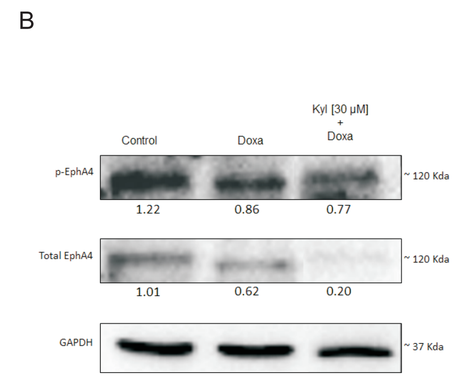


Fig. 3. HT-29 cells were preteated for 1 hr with kyl (30 µM) and after with doxasozin (23 µM) the cells were maintained for 24h. (A) Cell morphology analyzed using phase-contrast microscopy. Scale bar 50 µm. (B) Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels. GAPDH protein was used as a loading control. Numbers below the figure represent the ratio of band's optical density as fold change of protein expression normalized by GAPDH. Doxa: doxazosin

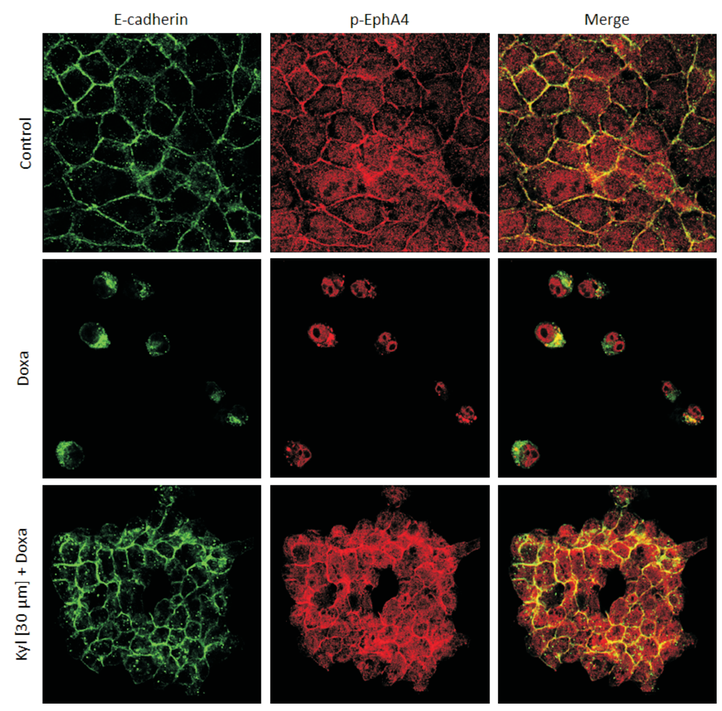
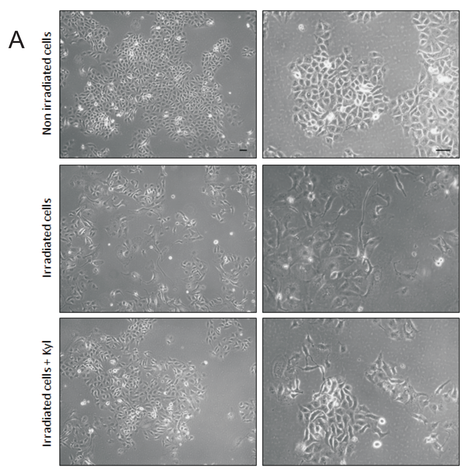


Fig. 4. HT-29 cells were preteated for 1 hr with kyl (30 µM) and after with doxasozin (23 µM) the cells were maintained for 24h. Cells were grown in glass coverlips until colony formation and subjected to immunofluorescence analysis. The nucleus was stained with DAPI. Doxa: doxazosin



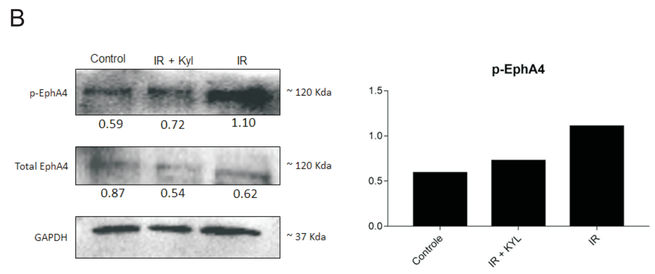


Fig. 5. HT-29 F1 cells, the group Irradiated + kyl were pretreated for 1 hr with 30 µM of Kyl and submeted to irradiation. (A) Cells morphology of the HT-29 F1 cells analyzed using phase-contrast microscopy. Scale bar 50 µm. (B) Western blot analysis of p-EphA4 (Tyr-602) and EphA4 protein levels. GAPDH protein was used as a loading control. Numbers below the figure represent the ratio of band's optical density as fold change of protein expression normalized by GAPDH.

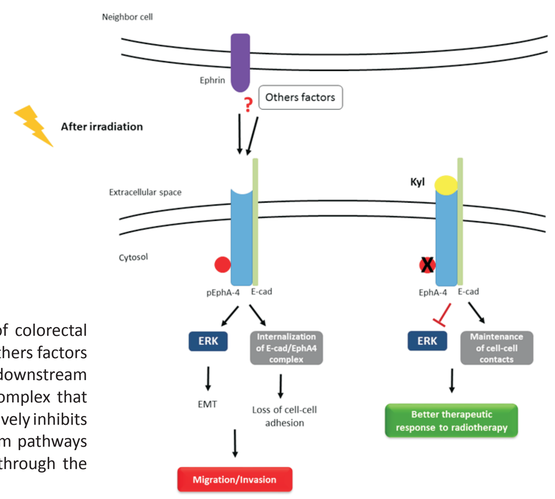


Fig. 6. Illustration of the signaling pathways induced in the progeny of colorectal cancer and the possible mechanism of action of Kyl. Ephrins as well as others factors can activated the EphA4 phosphorylation which leads to activation of downstream molecules such as ERK and the internalization of E-cadherin/EphA4 complex that culminates in migration and invasion (left). Kyl is a molecule that competitively inhibits Ephrin binding to EphA4 receptor and theoretically inhibits downstream pathways activated by EphA4 and allows the maintenance of cell-cell contacts through the retention of E-cadherin in the membrane (right).

REFERENCES

BASTOS, L. G., DE MARCONDES, P. G., DE-FREITAS-JUNIOR, J. C., LEVE, F., MENCALHA, A. L., DE SOUZA, W. F., DE ARAUJO, W. M., TANAKA, M. N., ABDELHAY, E. S. & MORGADO-DÍAZ, J. A. 2014. Progeny from irradiated colorectal cancer cells acquire an EMT-like phenotype and activate Wnt/β-catenin pathway. *J Cell Biochem*, 115, 2175-87.
JEMAL, A., BRAY, F., CENTER, M. M., FERLAY, J., WARD, E. & FORMAN, D. 2011. Global cancer statistics. *CA Cancer J Clin*, 61, 69-90.
GLYNNE-JONES, R., HADAKI, M. & HARRISON, M. 2013. The status of targeted agents in the setting of neoadjuvant radiation therapy in locally advanced rectal cancers. *J Gastrointest Oncol*, 4, 264-84.
MARCONDES, P. G., BASTOS, L. G., DE-FREITAS-JUNIOR, J. C., ROCHA, M. R. & MORGADO-DÍAZ, J. A. 2016. EphA4-mediated signaling regulates the aggressive phenotype of irradiation survivor colorectal cancer cells. *Tumour Biol*, 37, 12411-12422.
MARCONDES, P. G. & MORGADO-DÍAZ, J. A. 2017. The Role of EphA4 Signaling in Radiation-Induced EMT-Like Phenotype in Colorectal Cancer Cells. *J Cell Biochem*, 118, 442-445.
SAUER, R., BECKER, H., HOHENBERGER, W., RÖDEL, C., WITTEKIND, C., FIETKAU, R., MARTUS, P., TSCHMELITSCH, J., HAGER, E., HESS, C. F., KARSTENS, J. H., LIERSCH, T., SCHMIDBERGER, H., RAAB, R. & GROUP, G. R. C. S. 2004. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*, 351, 1731-40.
VICINI, F. A., KESTIN, L., HUANG, R. & MARTINEZ, A. 2003. Does local recurrence affect the rate of distant metastases and survival in patients with early-stage breast carcinoma treated with breast-conserving therapy? *Cancer*, 97, 910-9.

Financial Support

CAPES · FAPERJ · CNPq

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA