

Genetic variants and expression of AEG-1 in relation to clinical outcome and radiotherapy response in colorectal cancer patients and cell lines

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Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide. The therapy of CRC has been critically improved during the past two decades, but the treatment response varies significantly between different treatments and patients. Therefore it is necessary to search for biomarkers related to more suitable prognosis and treatment.

Astrocyte elevated gene-1 (AEG-1) is markedly overexpressed in many types of cancers, and is a downstream target of Ha-ras signaling. Upon expression, AEG-1 can activate several oncogenic signalling pathways such as PI3K/Akt, MAPK, Wnt, RNA interference and NF- κ B which are involved in crucial aspects of tumor biology including apoptosis, invasion, metastasis, angiogenesis, and chemoresistance.

The aim of this study was to evaluate the prognostic and predictive value of AEG-1, in terms of:

- genetic variants
- mRNA and protein expression

Materials and Methods

We analyzed genetic variants as well as the mRNA and protein expression of AEG-1 in 593 CRC patient samples by direct Sanger sequencing, qPCR and immunohistochemistry (IHC). 158 patients in the patient group were rectal cancer patients enrolled in the Swedish clinical trial of preoperative radiotherapy (RT). AEG-1 expression in response to γ -irradiation was analyzed by Western blot in 5 colon cancer cell lines and the clonogenic survival, after AEG-1 knock-down and irradiation, was measured.

Results

We found 29 variants, of which 12 were novel. Six of the variants were exonic and 23 intronic. It was shown before that the variants c.1353G>A, rs2331652 and c.1679-6 T>C were connected to breast cancer susceptibility but we found them only in 4, respectively in 1 sample. AEG-1 mRNA and protein expression, revealed significantly increased expression in the primary tumors and metastases compared to the normal mucosa.

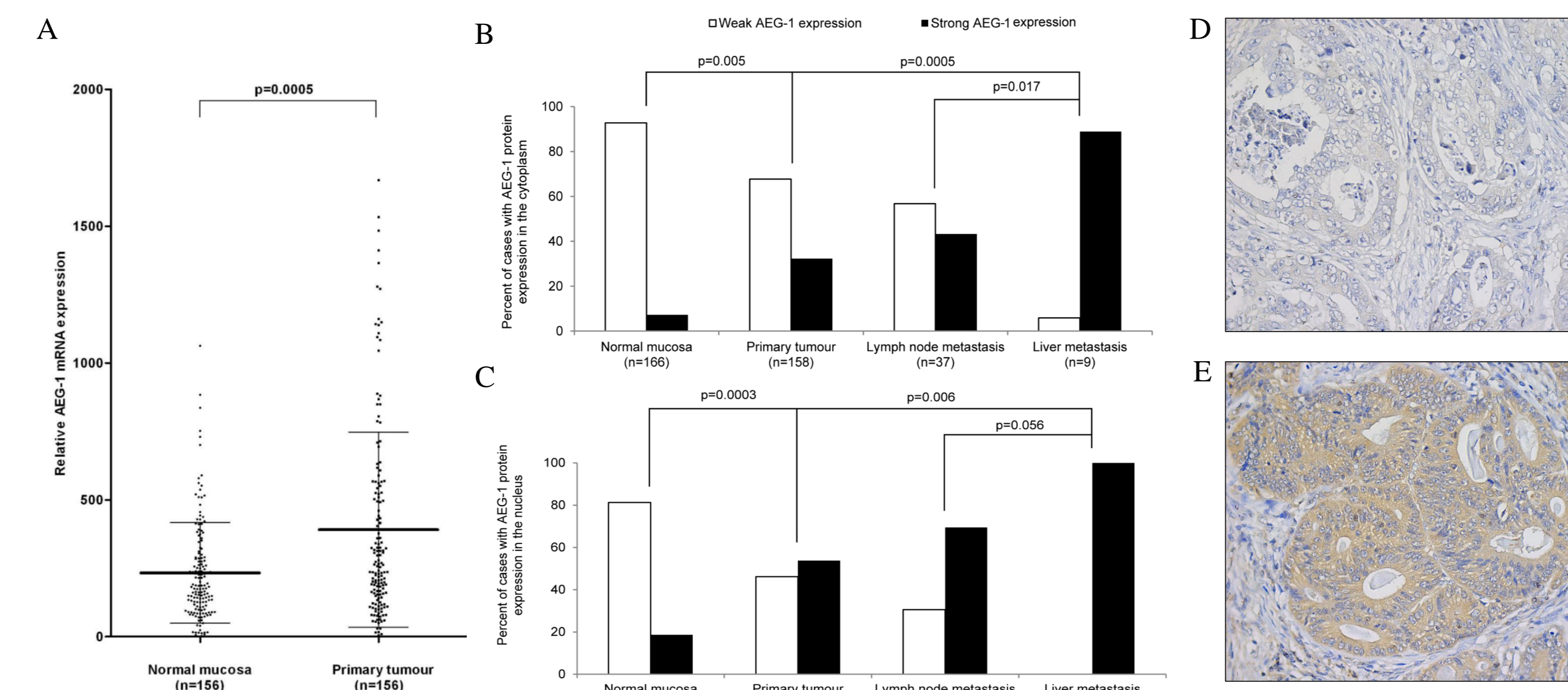


Figure 1: AEG-1 mRNA (A) and protein (B,C) expression increased during CRC development. Low (D) and high (E) IHC staining of AEG-1 in the primary tumor (400x).

In rectal cancer patients from the Swedish trial of preoperative RT, high AEG-1 expression correlated with higher risk of distant recurrence and disease free survival ($p=0.009$ and $p=0.007$, respectively) only in patients receiving preoperative RT, independently of the tumor stage.

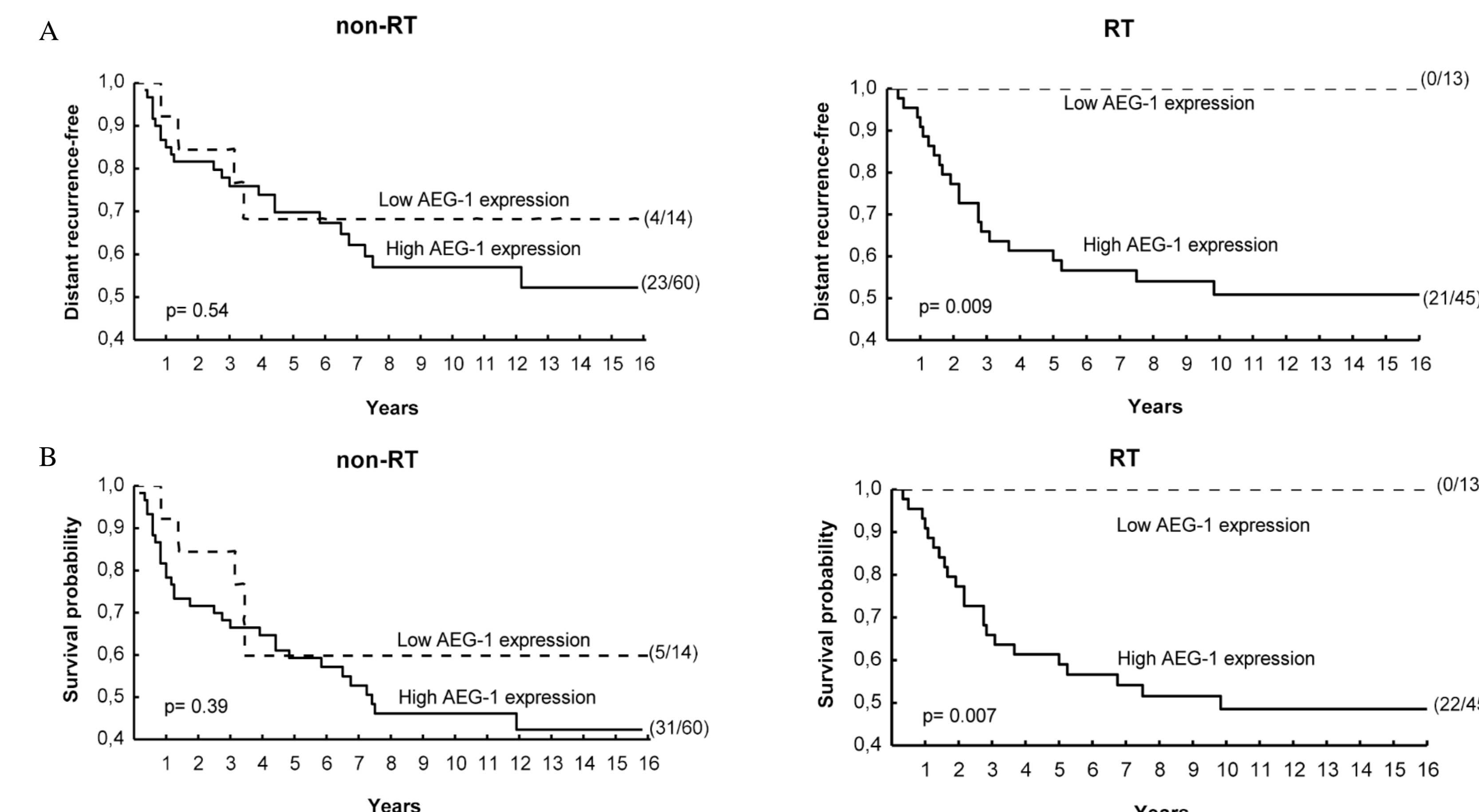


Figure 2: High AEG-1 expression correlated with a higher risk of distant recurrence (A) and disease free survival (B), only in patients receiving preoperative RT.

The AEG-1 expression was up-regulated shortly after 2 Gy γ -radiation in KM12C, KM12L4a and SW480, but not in SW620 and HCT116.

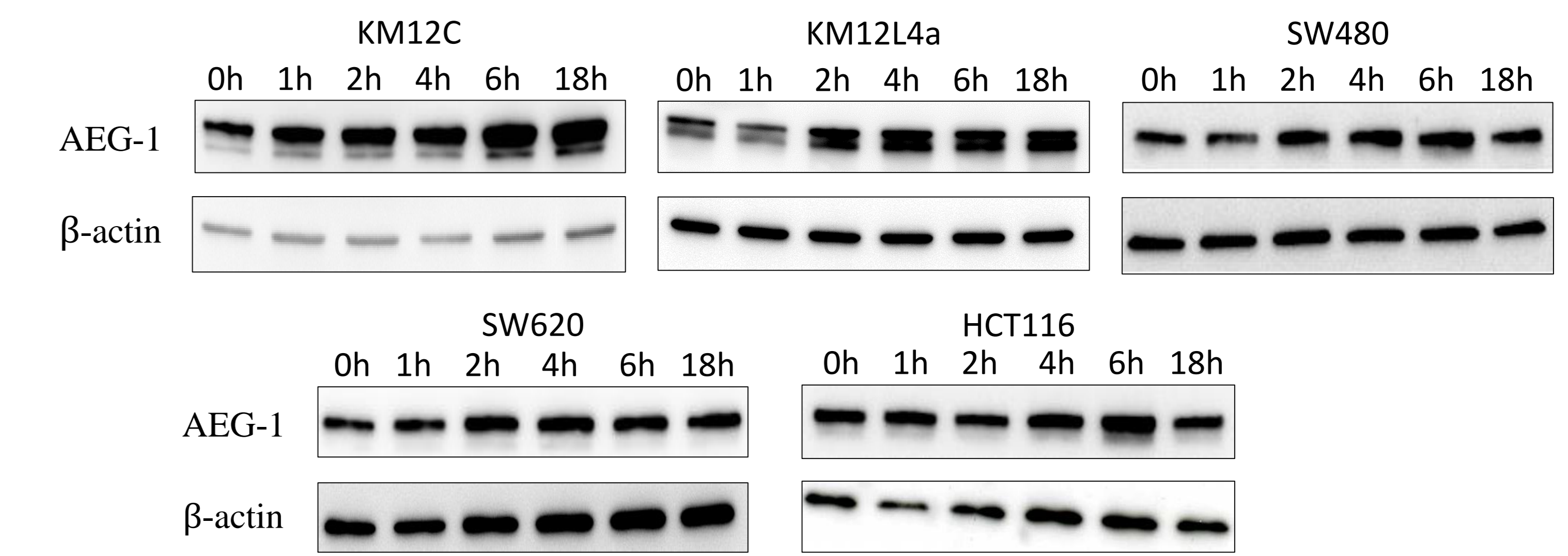


Figure 4: AEG-1 expression increased shortly after 2 Gy γ -radiation in the cell line KM12C, KM12L4a and SW480, but not in SW620 and HCT116.

AEG-1 knock-down in the radioresistant cell lines KM12L4a, SW480 and SW620 resulted in reduction of the survival after radiation, but not in the radiosensitive cell lines KM12C and HCT116.

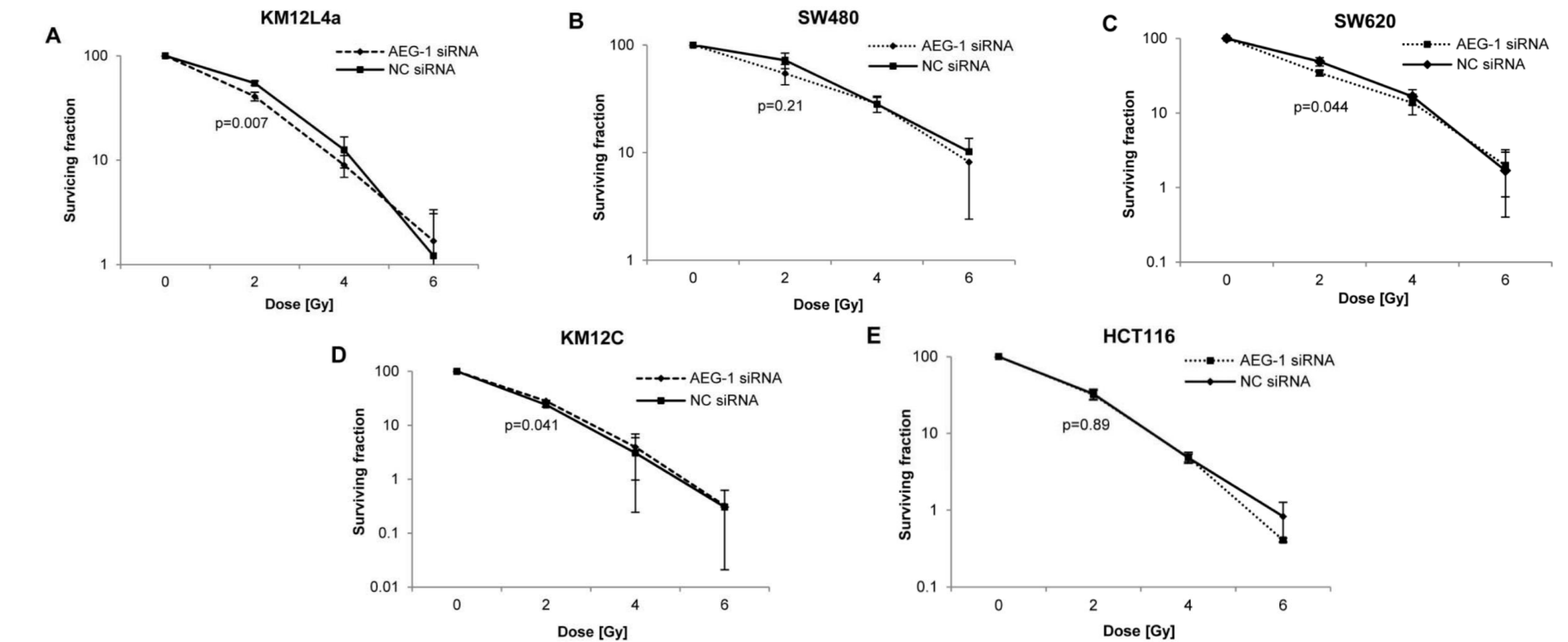


Figure 3: AEG-1 knock-down decreased the clonogenic survival after 2 Gy radiation in the cell lines KM12L4a, SW480 and SW620, but not in the cell lines KM12C and HCT116.

Conclusions

- AEG-1 expression correlates with CRC development and metastasis
- AEG-1 protein expression is an independent prognostic factor for distant recurrence and disease-free survival in rectal cancer patients after preoperative RT treatment
- AEG-1 is a possible radiosensitizing target for rectal cancer

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