Introduction

Ewing sarcoma (ES) is an aggressive solid bone and soft tissue malignancy of children and young adults. ES is originated from nonrandom chromosomal translocations that yield in-frame fusion of the amino terminus of the EWS gene on chromosome 22 and the carboxyl terminus of a member of the ETS family of transcription factors. There are several EWS-ETS aberrant transcription factors of which EWS-FLI1 fusions are the most frequent[1]. The fusion protein EWS-FLI1 plays a role in different aspects of tumor development, maintenance, and progression through the regulation of EWS-FLI1 target genes. Some of most relevant EWS-FLI1 target genes identified up to date are NKX2-2, cholecystokinin[2], MRH38[3], TGFBR2[4], IGF2BP[5] and LGUN[6] among others. Identification of EWS-ETS target genes and their role in tumor development will facilitate the translation into new treatment modalities for these diseases.

In this work we identified a new target of EWS-FLI1 oncogene; Sprouty 1 (SPRY1). SPRY1 is an upstream antagonist of BAG5 and it has been suggested that it may have a tumour suppressor function since its expression is decreased in human cancer, including tumors from breast, prostate, and liver tissue[7].

Objectives

1. Identify new EWS-FLI1 target genes.
2. Study the mechanism involved in the regulation of SPRY1 expression.
3. Analyse the functional relevance of SPRY1 in Ewing Sarcoma.

Results

SPRY1 expression is downregulated by EWS/FLI1 in Ewing sarcoma cells

Figure 3. SPRY1 expression is downregulated by EWS/FLI1 in Ewing sarcoma cells.

Figure 4. HDACs are partially involved in the repression of SPRY1.

SPRY1 is not expressed in Ewing Sarcoma cells

Figure 5. SPRY1 is not expressed in Ewing sarcoma cells.

Establishment of Ewing sarcoma cells expressing inducible SPRY1

Figure 6. Inducible expression of SPRY1 in Ewing sarcoma cells.

SPRY1 induction in Ewing sarcoma cells affects cell proliferation

Figure 7. SPRY1 induction in Ewing sarcoma cells.

Conclusions

1. SPRY1 expression is strongly downregulated by EWS/FLI1 in Ewing sarcoma cells.
2. These effects are partially mediated by epigenetic mechanisms.
3. Induction of SPRY1 expression in Ewing sarcoma cells line moderately inhibits cell proliferation.
4. SPRY1 regulates MAPK pathway by inhibiting ERK phosphorylation.

Acknowledgments

This study was supported by grants of the Ministerio de Ciencia e Innovación (SAF2007-67359) and the Associate Public-Private center of cancer infantil.

Materials and Methods

Establishment of Ewing Sarcoma cell lines stably expressing doxycycline-inducible EWS/FLI1 shRNA or SPRY1 cDNA. Ewing A673, SKES, and SKMCC cells were infected with a plasmid containing the tetracycline repressor and afterward with a plasmid containing EWS/FLI1 shRNA or SPRY1 cDNA regulated by doxycyclin. When these cells are treated with doxycyclin at a concentration of 1 μg/ml, they express the EWS/FLI1-specific shRNA, which subsequently knockdown EWS/FLI1 levels, or the SPRY1 mRNA.

Quantitative reverse transcription-polymerase chain reaction (RT-qPCR): Reactions were run on a BioRad iCycler (Bio-Rad Laboratories) using specific Taqman probes for the genes analyzed. Relative expression was calculated by 2−ΔΔCt method.

Western blot analysis and antibodies: Following electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Millipore), then blocked with 5% milk and incubated with primary antibodies. Finally they were incubated with horseradish peroxidase-conjugated secondary antibodies and subjected to chemiluminescence detection analysis.

Promotion assay: 5×10^4 cells were plated in presence or absence of doxycyclin for 4-5 days. Then they were trypsinized, counted and regulated for a minimum of 10 days.

Chemical Agents: Trichostatin A (TSA) (Sigma), Vorinostat (SAHA) (Sigma), S-2,7-deoxycytidine (Sigma) and Valproic Acid (VPA) (Sigma) were dissolved in DMSO first and then further dissolved in PBS until desired concentration. bFGF (PeproTech) was used at 10ng/ml; IGF1 (PeproTech) at 5ng/ml or in PBS.

Bibliography