Introduction

Ewing sarcoma (ES) and peripheral primitive neuroectodermal tumors (PNET) are associated with a chromosomal translocation, resulting in a fusion of the amino-terminus of EWS with the DNA-binding domain of an ETS transcription factor (most commonly FLI1 or ERG). Although previous reports suggested that these EWS-ETS chimera proteins would act as aberrant transcription factors, their downstream targets have not been fully elucidated. The objective of this study is to investigate target genes of these EWS-ETS fusion proteins.

Methods

To identify downstream targets of these chimera proteins, we first established HT-1080 (human fibrosarcoma cell line) cell populations expressing EWS-ETSs (EWS-ERG and EWS-FLI1) or their normal ETSs counterparts (ERG and FLI1) using retrovirus vectors, pLNCX and pBabe-Puro (Figure 1). Gene expression patterns in each pool of HT-1080 transfектants were compared in cDNA microarray analysis with a duplicate set of 23040 cDNA clones. Upon the first screening using cDNA microarray, the genes up-regulated by EWS-ETS fusion proteins but not by normal ETSs proteins were subjected to the second screening using northern and western blot analysis. The putative target genes for EWS-ETSs identified by these analyses were further examined for their expression by inhibition experiments using an antisense cDNA expression vector (pIRESneo2), and by northern and western blot analysis using six ES cell lines and tissue sections of six primary ES tumors. Also, the transcription of the putative target gene was determined by promoter-reporter gene assay.

Results

The cDNA microarray analysis and subsequent northern and western blot analysis revealed that Tenascin-C (TN-C) is induced to much higher levels in HT-1080 cells overexpressing EWS-ETSs than in cells overexpressing normal ETSs by two distinct expression vectors. Furthermore, an antisense cDNA expression vector for TN-C reduced the expression of both endogenous TN-C mRNA and protein coordinately with attenuation of EWS-FLI1 fusion protein expression. A TN-C promoter-reporter gene assay revealed that EWS-ETS fusion proteins up-regulate the transcription of TN-C gene in both a direct and indirect manner. High levels of TN-C expression were observed in a subset of ES cell lines (3 of 6) and primary tumor tissues (4 of 6).

Discussion

Using cDNA microarray, we screened 23040 cDNA clones and identified TN-C as a putative target gene for EWS-ETSs fusion genes according to its selective upregulation associated with overexpression of EWS-ETSs. TN-C was upregulated not only in HT-1080 fibrosarcoma cell lines overexpressed for EWS-ETSs but also in ES cell lines and ES tumor tissues. Moreover, EWS-ETS fusion proteins promoted the transcription of TN-C. These findings suggest that TN-C may be a downstream target of EWS-ETSs in ES and PNET.

TN-C is an extracellular matrix protein. The expression of TN-C is precisely regulated, both temporally and spatially, and is observed during fetal development. TN-C is not expressed in normal adult tissues, but it is activated under many pathological conditions including wound healing, inflammation and malignancies. It has been reported that TN-C in tumors is typically expressed in stroma, and TN-C has many biological activities, such as tumor cell proliferation, invasion, metastasis and induction of cell rounding in tumor-matrix interactions in other tumor types. Our data suggest that TN-C might also promote these activities in ES and PNET following transcriptional up-regulation by EWS-ETS fusion genes.

Figure 1. Expression of the transfected genes in HT-1080 cells.

A. Northern blot analysis revealed expression of the appropriate mRNA in a pool of HT-1080 cells transfected with a control empty vector (lane 1), ERG (lane 2), EWS-ERG (lane 3), FLI1 (lane 4) and EWS-FLI1 (lane 5), respectively. 10 ?g of total RNA extracted from each cell pool was loaded. Hybridization was performed with a human ERG or FLI1 cDNA fragment as a probe.

B. Western blot analysis showed expression of the appropriate protein in a pool of HT-1080 cells transfected with a control vector (lanes 1 and 4), ERG (lane 2), EWS-ERG (lane 3), FLI1 (lane 5) and EWS-FLI1 (lane 6), respectively. 20 ?g of extracted whole protein lysate was loaded in each lane. Each protein was detected with an antibody against the C-terminus region of ERG or FLI1.