IDENTIFICATION OF LAMININ BETA3 ISOFORMS DOWNSTREAM OF EWS-ETS FUSION GENES IN EWING FAMILY TUMORS

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Introduction
Ewing family tumors (EFTs) are associated with a specific 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.

Materials and Methods
Human fibrosarcoma cells expressing EWS-ETs (EWS-ERG and EWS-FLI1) or their normal ETs counterparts (ERG and FLI1) were established from HT1080 cells using retrovirus vectors, pLNCX and pBabe-Puro. Gene expression patterns in each pool of HT-1080 transfectants were compared in cDNA microarray analysis with a duplicate set of 23040 cDNA clones. Genes up-regulated in EWS-ETS transfectants but not in normal ETs transfectants in cDNA microarray as well as Northern and Western blot analysis were regarded as putative target genes for EWS-ETs. Of these, a gene coded as LAMB3 was further analyzed by (1) inhibition experiments using antisense cDNA expression vector (pIRESneo2) for EWS-FLI1, and (2) Northern and Western blot analysis in various sarcoma cell lines, Ewing sarcoma specimens.

Results
The cDNA microarray analysis and subsequent Northern and Western blot analysis revealed that Laminin beta3 gene (LAMB3) was induced to much higher levels in EWS-ETS transfectants than normal ETs transfectants (Fig. 1). An antisense cDNA expression vector for EWS-FLI1 reduced the expression of both endogenous LAMB3 mRNA and protein coordinately with attenuation of EWS-FLI1 fusion protein expression in EWS-ETS transfectants. All human sarcoma cell lines tested expressed LAMB3 (Fig. 2). Notably, Ewing sarcoma cell lines expressed smaller sizes of LAMB3 transcripts in addition to the wild type, whereas other sarcoma cell lines expressed only wild type LAMB3. Cloning of those smaller sizes of LAMB3 transcripts from the cDNA library of a Ewing sarcoma cell line, WES, revealed nine isoforms of LAMB3. Those isoforms were expressed in Ewing sarcoma tissues.

Discussion
Using cDNA microarray, we previously defined Id2 and Tenascin-C as targets of transcriptional activation by EWS-ETS proteins in Ewing family tumors (1,2). In the present study, we additionally identified Laminin beta3 by the same approach. LAMB3 is a subunit of extracellular matrix protein, Laminin-5 (alpha3, beta3, gamma2). While various intracellular proteins have been defined as EWS-ETS target genes, our results regarding LAMB3 and Tenascin-C support the involvement of extracellular matrix proteins in oncogenesis of EFTs. Furthermore, LAMB3 was expressed in Ewing sarcoma cell lines and tissues in a variety of isoforms. Selective expression of LAMB3 isoforms in Ewing sarcomas suggest their specific oncological as well as physiological roles.

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References