Gene Expression Patterns 10 (2010) 127–134

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Expression of two novel transcripts in the mouse definitive endoderm

Ali S. Hassan [a,b], Juan Hou [a], Wei Wei [a], Pamela A. Hoodless [a,b,*]

[a] Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada
[b] Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

ARTICLE INFO

Article history:
Received 5 August 2009
Received in revised form 3 February 2010
Accepted 5 February 2010
Available online 12 February 2010

Keywords:
Mouse
Embryo
Gastrulation
Definitive endoderm
Node
Posterior notochord
Serial analysis of gene expression
SAGE
AK014119
AK084355
Ende
Npe
Pyy
Hex

ABSTRACT

Here we describe the expression of two novel transcripts, *Ende* (AK014119) and *Npe* (AK084355), during early mouse embryogenesis. *Ende* mRNA was first detected at embryonic day (E) 7.0 in a small population of epiblast cells in the distal half of the embryo. At E7.5, *Ende* was expressed by newly formed definitive endoderm cells in the proximal half of the embryo, and was not expressed in extra-embryonic endoderm. This expression pattern then changed to the ventral aspect of the developing foregut pocket and the entire hindgut pocket at E8.0–8.5, before becoming restricted to the foregut overlying the heart and the posterior-most hindgut. By E9.25 *Ende* expression was also observed in the posterior half of the ventral neural tube. Thus, *Ende* was expressed dynamically and in specific populations of the definitive endoderm from E7.0 to E8.5. We found *Npe* expression to be restricted to the node/posterior notochord region at the distal tip of the embryo between E7.0 and E8.0. By E9.5, *Npe* expression was observed in the posterior-most population of dorsal hindgut cells and notochord cells. Given their expression in mouse definitive endoderm populations, *Ende* and *Npe* will be valuable tools to study formation and development of this tissue.

1. Results and discussions

The definitive endoderm is one of the three germ layers of the embryo that are generated during gastrulation and gives rise to the organs of the respiratory and gastrointestinal tracts including the lungs, liver, pancreas and intestine (Wells and Melton, 1999). Progress in the understanding of definitive endoderm formation and patterning has lagged behind that of the mesoderm and ectoderm, particularly due to the lack of genetic markers specific to this tissue. Understanding the development of the definitive endoderm is crucial for future *in vitro* based approaches to regenerative medicine for diabetes or liver diseases.

We previously described a systematic screen for genes expressed in the definitive endoderm using serial analysis of gene expression (SAGE) libraries (Hou et al., 2007). Three mouse definitive endoderm LongSAGE libraries were compared against more than 200 mouse LongSAGE libraries from various embryonic stages and tissues, successfully identifying several genes as new markers for the definitive endoderm, including *Nephrocan* (*Nepn*) and *Peptide YY* (*Pyy*) (Hou et al., 2007). This study, however, limited itself to screening for sequences that mapped to transcript databases, and omitted a list of non-annotated, uncharacterized sequences, potentially representing novel genes enriched for expression in the definitive endoderm. Thus, we extended this SAGE study and analyzed tag sequences representing non-annotated transcripts to identify candidates expressed in the definitive endoderm. From this analysis we identified two ESTs, AK014119 and AK084355, showing enrichment for definitive endoderm. AK014119 was renamed *endoderm enriched* or *Ende* and AK084355 was renamed *notochord posterior end* or *Npe* for the sake of simplicity.

Ende lies on chromosome 8 and is 9.8 kb upstream of *Trim60* and 48 kb downstream of a non-coding RNA, EST BC030870. *Ende* has a predicted hypothetical protein from a short 240 bp open reading frame (http://www.ncbi.nlm.nih.gov/nuccore/74182885). Rapid amplification of 5′ cDNA end (5′ RACE) was done to define the start of the transcript in E8.5 mouse embryo. The RACE product for *Ende* indicated that the transcript begins in exon 2 of the annotated gene model at nucleotide +246, although transcriptome sequencing of purified hepatoblasts suggested that the 5′ end of

* Corresponding author. Address: Terry Fox Laboratory, British Columbia Cancer Agency, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3. Tel.: +1 604 675 8133; fax: +1 604 877 0712.
E-mail address: hoodless@bccrc.ca (P.A. Hoodless).

1567-133X/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2010.02.001

the transcript may extend into intron 1 (Supplementary Fig.). The RACE result was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) (data not shown). Interestingly, we did not find any evidence that the predicted first exon is part of the transcript that is expressed in E8.5 mouse embryo or purified hepatoblasts.

Npe lies on chromosome 12 and is 68 kb downstream of an EST DV039614, and 217 kb upstream of *Sp4*, and lacks a definitive open reading frame (NCBI ORF finder, http://pga.mgh.harvard.edu/web_apps/web_map/start). We were unable to generate a RACE product for *Npe*, likely due to the highly restricted and lower level of expression compared to *Ende*. Since *Npe* has no definitive coding region and displays no homology to known classes of non-coding RNAs such as tRNA and rRNA, the transcript was bioinformatically folded to the most thermodynamically stable secondary structure using mFOLD (http://mfold.bioinfo.rpi.edu/; Zuker, 2003). Short hair-pin loops from the secondary structure were then analysed with a miRNA precursor prediction program MiPred (http://www.bioinf.seu.edu.cn/miRNA/; Jiang et al., 2007) to explore the potential of *Npe* as a miRNA gene. The algorithm predicted several potential miRNA precursors along the transcript suggesting *Npe* maybe a non-coding miRNA gene (data not shown). *Ende* was also put through the same analysis, but did not produce any predicted miRNAs. More studies will be needed to explore the coding or non-

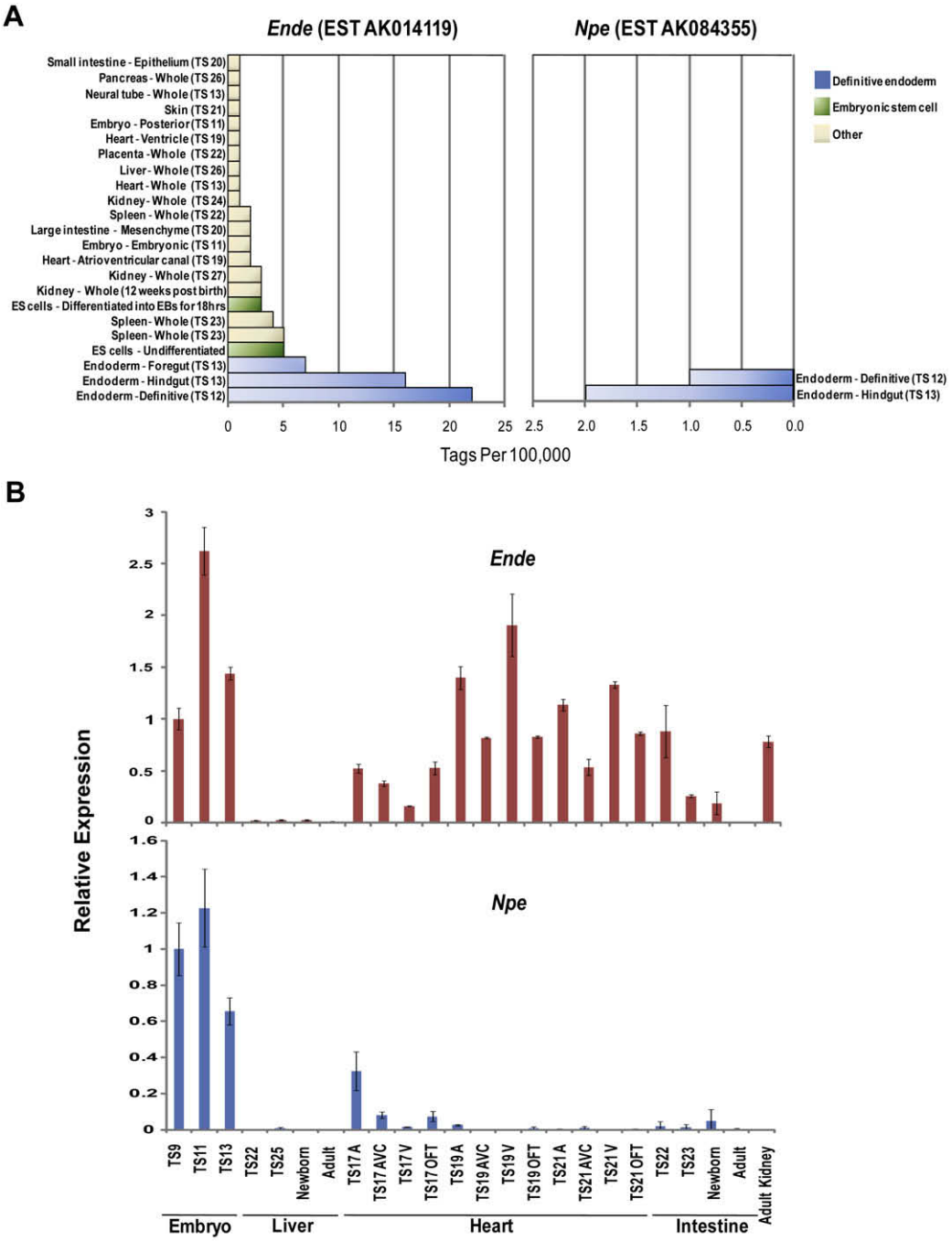

Fig. 1. Expression of *Ende* and *Npe* during mouse embryogenesis. (A) Transcript distribution for *Ende* and *Npe* in Mouse Atlas of Gene Expression SAGE libraries. Transcripts are represented as tag counts for *Ende* and *Npe*, normalized to library size and expressed as tags per 100,000 total tags. (B) qRT-PCR analysis of *Ende* and *Npe* during early post-implantation stages in the mouse embryo, the liver, heart, intestine and kidney. Theiler stage (TS), atria (A), ventricle (V), atrioventricular canal (AVC), outflow tract (OFT).