Indian Journal of Animal Sciences **81** (11): 1141–1142, November 2011

Relative expression of an "H19 like" gene in stomach and intestine of mouse

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Received: 1 April 2011; Accepted: 21 May 2011

Key words: Gene expression, H19 gene, Intestine, Mouse

Several non-coding RNAs have been identified recently, in the regulation of gene expression. H19 is one of the genes that codes for long non-conding RNA (Brunkow and Tilghman 1991) and is transcribed by RNA polymerase II, spliced and polyadenylated, but it does not appear to be translated (Brannan *et al*. 1990). The H19 gene is unique, and is only transcribed from the maternally inherited allele; the paternal H19 allele is not expressed (Rachmilewitz *et al*. 1992). The H19 is expressed strongly during embryogenesis (Matouk *et al*. 2007) and is an imprinted oncofetal gene. The expression is reduced or is absent in most of the adult tissues. The over expression of H19 gene has been observed in cancer cells (Lottin *et al*. 2002) and implicated for tumor metastasis (Yang *et al*. 2004). H19 expression has also been associated with carcinogen induced tumorigenesis (Elkin *et al*. 1998).

The epithelial cell lining of the gastrointestinal tract is one of the most rapidly proliferating tissues in the body. The constant state of renewal of differentiated epithelial cells is sustained by a continual supply of progeny from multipotent progenitors that originate from stem cells located within the intestinal crypts. In addition to supporting normal epithelial homeostasis, intestinal stem cells (ISC) are thought to play an important role in the rapid expansion of the gut during development, tissue regeneration following injury or surgical loss, and malignancy (Bach *et al.* 2000, Garrison *et al.* 2009). However the proliferation of stem cell populations is tightly regulated by several poorly understood mechanisms to prevent them from becoming malignant. The relation between loss of imprinting of insulin like growth factor-II, H19 gene expression and development of colorectal cancer in murine (Sakatani *et al.* 2005) has been demonstrated. An inverse relationship between the H19 gene expression in stem cell de-differentiation and tumorigenesis has been reported (Scott *et al.* 2005). In the present study whether gastrointestinal tract (GIT) epithelial cells express H19 gene in adult mice was investigated, which will help to understand the possible mechanisms in development of gut tumors.

Tissue isolation and real time polymerase chain reaction: Adult albino male mice of IVRI strain (n,6), obtained from Laboratory Animal Resource Section of Indian Veterinary Research Institute was used for the study. The animals were humanly sacrificed in the laboratory and the tissues were collected in QIAzol reagent. The tissues were processed immediately after sacrifice, and total RNA was isolated using QIAzol reagent. One micrograms of total RNA was reverse transcribed using oligo$(dT)_{18}$ primers and Murine Monloney Leukemia Virus reverse transcriptase in the presence of RNAse inhibitor (40 units) as per manufacturers protocol. The real time reactions were performed with 100ng of reverse transcribed total RNA using Quantitect SYBR green master mix in a final volume of 20µl in a MX3005 instrument. The primers of H19 and beta actin (housekeeping gene) used for the study is shown in Table 1. Typical 40 cycles of amplifications consisted of the following thermal cycling conditions: initial denaturation- 95°C for 5 min cycling- 94°C for 30sec, 50°C for 45sec and 72°C for 60sec. The specificity of the amplification was confirmed from the dissociation curve of PCR product. The dissociation curve of the PCR product was accomplished initial heating at 95°C for 1 min followed by a gradient from 50°C for 30sec to 95°C for 30sec.

Table 1. Primer sequences used for real time polymerase amplification of genes

Primer/gene	H19	Beta actin
Forward (5' to 3')	AGCCAGGCATTCATCCCGGT	CGCACCACTGGCATTGTCAT
Reverse (5' to 3')	AGCGCTGTCTTCACCTGC	TCCAAGGCGACGTAGCAGAG

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The relative quantification of H19 gene expression in tissues was calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The threshold cycle (Ct) values were based on

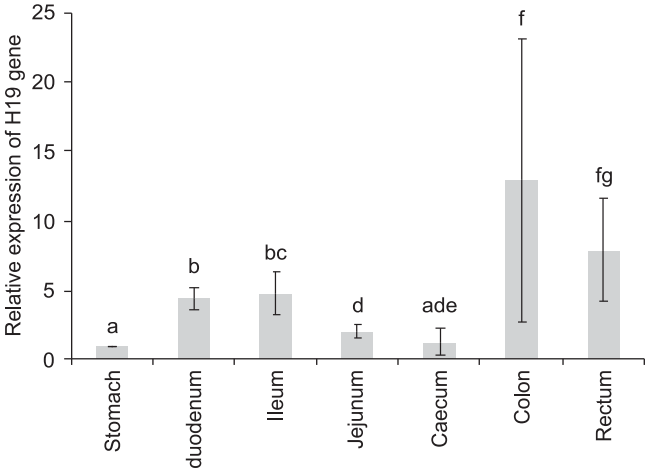

Fig 1. The relative expression of H19 like gene in stomach, small and large intestine. The graphs with same superscript did not differ significantly (P>0.05).

duplicate measurement. The quantification values obtained for stomach tissue were arbitrarily set to 1.0 and relative expression of H19 in various intestinal regions were calculated. The data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

In the present study a gene amplified by primers designed for H19 gene could be demonstrated in stomach and various intestinal regions (Fig. 1). The expression in colon and rectal region was highest. Lowest expression levels were seen in caecum and stomach regions. The expression of gene in the small intestine was between stomach and large intestine. Since the expression of H19 gene is limited in adult tissues (Brunkow and Tilghman 1991), and a transcript could be detected which could be amplified by the primers for H19 gene, it will be ideal to denote as H19 like, rather than H19 itself. To confirm the identity of transcript as H19, isolation of complete RNA and nucleotide sequencing is required. The higher incidence of tumors of the large intestine over small intestine and stomach has been documented (National Cancer Institute 2011). Further studies are required to understand the relationship between higher transcript concentration of H19 like gene reported in the present study in large intestine over other regions of GIT in relation with stem cell proliferation and development of carcinogenesis.

SUMMARY

In the present study expression of a H19 like gene in the stomach, small and large intestine was studied. Higher transcript concentration of this gene was observed in large intestinal regions over other regions of GIT. The present study could not, however, establish the identity of transcript as H19 itself since nucleotide sequencing was not done. Since the expression of H19 gene with development of tumors and stem cell is known, further studies are required to establish the expression of the gene presently studied and the higher incidence rate of large intestinal tumors in comparison to stomach and small intestine.

ACKNOWLEDGEMENTS

The authors are thankful to Directors of Indian Veterinary Research Institute, Izatnagar and NRC on Pig, Guwahati for providing facilities for the study. Thanks are due to Dr. G. Sai Kumar for assistance in conducting RT-PCR reactions.

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