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Claudin profiling in the mouse during postnatal intestinal development and along the gastrointestinal tract reveals complex expression patterns

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Abstract

Members of the claudin protein family are key regulators of tight junction selectivity and are implicated in influencing development and cellular differentiation in the intestine and other tissues. The goal of the present study was to profile claudin gene expression and protein location during postnatal development of the mouse jejunum and in the adult mouse gut from duodenum to distal colon as a first step in understanding both normal claudin function and the pathologic implications of altered expression patterns. The relative expression of claudins 1–19 and other tight and adherens junction genes was determined by quantitative RT-PCR from six regions of normal mouse intestine and colon. Immunofluorescent localization was performed for claudins 1–5, 7, 8, 10, 12, 15, and 18. Transcripts for claudins 1–5, 7–13, 17, and 18 were all detected in adult intestine, although their relative abundance differed up to 1000-fold within individual segments. In contrast to the unchanging expression and localization of ZO-1, occludin, and JAM, most claudins were expressed in decreasing or increasing gradients or in more complex patterns along the longitudinal axis of the intestine and the crypt to villus/surface differentiation axis. During neonatal development at days 1, 14, 28, and 90 several claudins showed striking increases or decreases in transcript expression as well as changes in tissue localization along the crypt-villus axis. Claudin-19 was only detected at days 1 and 14. This database provides a resource for investigating regional and developmental differences in permselectivity, crypt to villus/surface differentiation and neoplastic changes along the gut and during postnatal development.

Keywords: Claudin; Claudins; Occludin; ZO-1; Cadherin; JAM; Intestine; Jejunum; Ileum; Cecum; Colon; Crypt; Villus; Epithelia; Endothelia; Tight junction; Differentiation; Postnatal development; Weaning; Epithelial transport; Quantitative PCR; Mouse

Claudins form a large family (>24 members) of transmembrane adhesion proteins located at intercellular tight junctions (Furuse et al., 1998; Nusrat et al., 2000; Schneeberger and Lynch, 2004; Van Itallie and Anderson, 2006). Their differential expression profiles are likely to underlie differences in electrical resistance and ionic charge selectivity observed among different epithelia (Colegio et al., 2002; Simon et al., 1999; Yu, 2003). Emerging evidence suggests claudins may also have a primary role in cell signaling and differentiation (Cheung et al., 2005; Soini, 2005; Swisshelm et al., 2005). Complex expression patterns have been documented during zebrafish development (Kollmar et al., 2001), and in *Drosophila*, claudins are required for proper tracheal epithelial morphogenesis (Behr et al., 2003; Wu et al., 2004). In cultured human cells cldn-1 induces transformation and activates Wnt β-catenin signaling (Dhawan et al., 2005).

The adult intestine undergoes continuous differentiation in a radial direction from stem cells in the crypt to the villus surface and varies in transport functions along the

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longitudinal axis from duodenum to distal colon (Binder, 1998). In addition, the intestine continues to develop from birth until after weaning (Henning, 1981). To investigate possible roles for claudins in: (a) regional differences in paracellular transport; (b) development; and (c) differentiation, we profiled the expression and localization of claudins 1–19 along the adult mouse intestine and in the jejunum during postnatal development.

1. Results and discussion

1.1. Claudin expression patterns in the adult mouse intestine

Quantitative real-time PCR (qRT-PCR) reveals that the adult intestine expresses many different claudins in complex quantitative and spatial patterns. Claudins are expressed variably along both the longitudinal axis and the crypt/villus differentiation axis (Figs. 1, 3 and 4 and Supplementary Fig. 3 and Table 1). In contrast, transcript levels for the TJ cytoplasmic protein ZO-1 do not vary relative to expression of Eef1a1, which was used as a general reference transcript. Therefore, in these studies claudin expression levels were normalized to ZO-1 expression. All primer pairs produced amplicons of the predicted size (Supplementary Fig. 1 and Table 1). Among the nineteen claudins tested, 1–5, 7–15, 17, and 18 were detected, while 6, 16, and 19 were not. The ability to detect all nineteen was confirmed in other tissues (Supplementary Fig. 1). Expression of other transmembrane tight/adherens junction genes, including occludin, JAM, and JAM-4 were very consistent from duodenum to distal colon, although cadherin showed a small but significant 2-fold increase from proximal to distal regions.

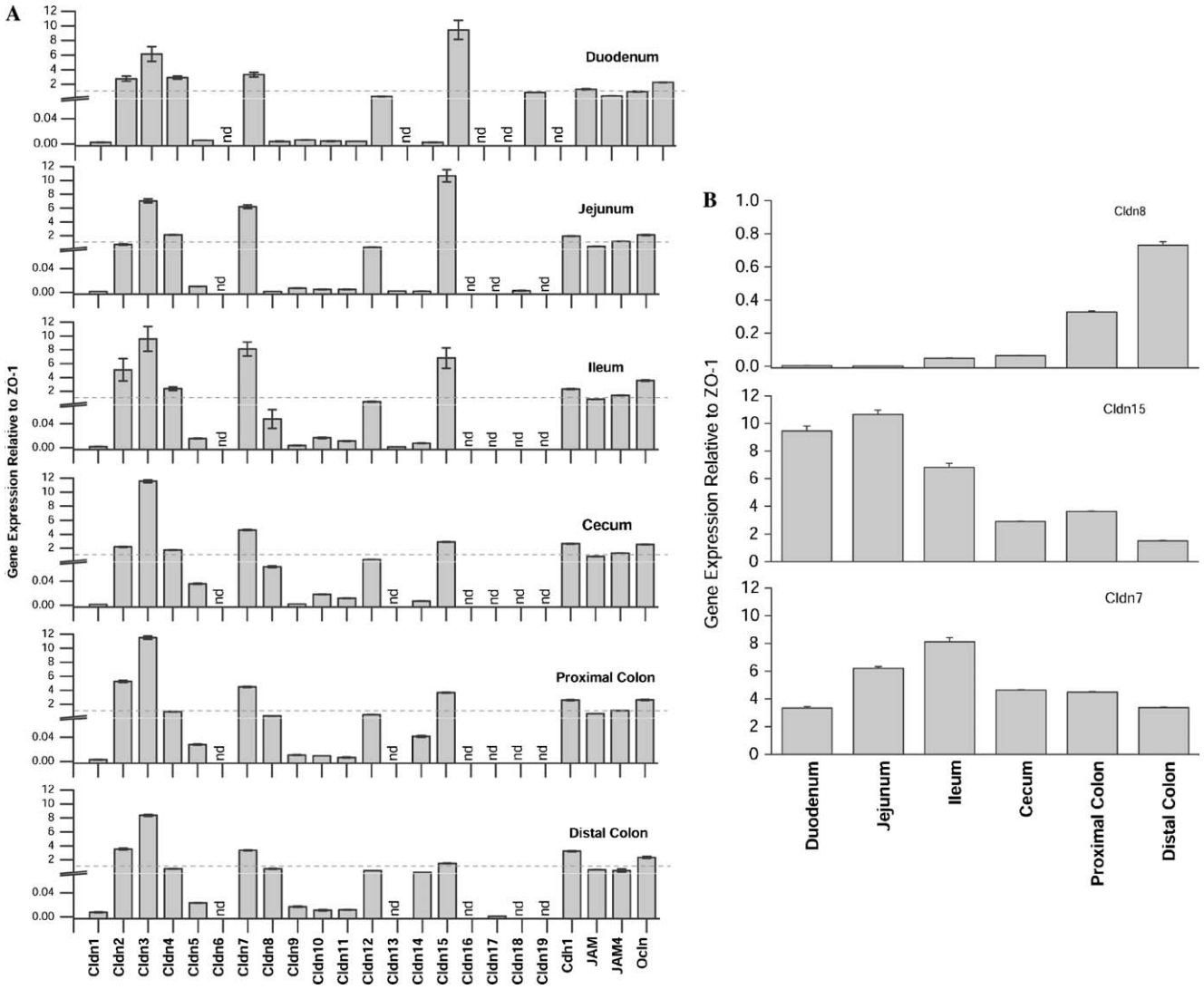

Fig. 1. (A) Gene expression profile by qReal Time-PCR of claudins 1–19, cadherin, JAM, JAM4, and occludin relative to ZO-1 (set as 1.0) in mouse duodenum, jejunum, ileum, cecum, proximal and distal colon. The ordinate axis is broken into two scales to accommodate the wide range in expression. Error bars = $[(2^{\%CV})/100] \times$ [relative expression]; nd, not detected. Expression levels vary by several thousand-fold among claudins within a single segment. Individual claudins can show gradients along the intestine. (B) Representative expression profiles illustrating patterns which increase (cldn-8), decrease (cldn-15) or show a more complex pattern (cldn-7) along the longitudinal axis of the gut.