Differential TLR Expression Profile in Both Epithelial Stem Cells (Epi-SC) Isolated From Each Segment of the Human Intestinal Tract and Primary Non-Transformed Epithelial Cell Lineages Derived from the Cultured Epi-SCs.

David A. Harrison, Shazia Ahmedi, and Asit Panja

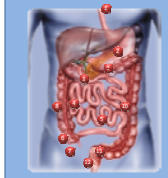
AlfaGene Bioscience, Inc.; Somerset, NJ, USA.

BACKGROUND

Toll-Like Receptors (TLRs) are transmembrane proteins which recognize pathogen associated molecular patterns (PAMPs) and activate the innate immune system against invading microbes. The luminal surface of the gastrointestinal epithelium is the largest area in the body and exposed to a myriad of microbial species on a constant basis. While site preference (small vs. large intestine) for many infectious organisms is well established, the expression profile and function of TLRs in various micro-environmentally differentiated regions of the GI tract has not been delineated. Altered TLR expression in intestinal epithelial cells cause or be indicative of aberrant immunity, infection, inflammation and/or malignant transformation in the GI tract.

The **AIM** of this study was to determine the expression levels of the five major TLRs (1, 2, 4, 5, 6, and 10) and their adaptor protein MyD88 in a novel cellular system of adult intestinal stem cells and differentiated epithelial lineages.

Isolation, culturing, and demonstration of stem cell characteristics of cells isolated from adult human GI tissues

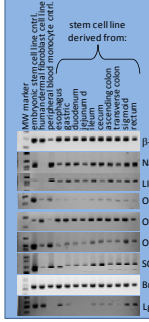


1 esophagus
2 stomach
3 duodenum
4 jejunum
5 ileum
6 cecum
7 appendix
8 ascending colon
9 transverse colon
10 decending colon
11 sigmoid
12 rectum

small intestine
large intestine

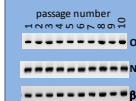
AlfaGene is able to isolate stem cells from adult GI tissues and derive differentiated epithelial cells from them. Stem cells from the esophagus, stomach, duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, sigmoid, and rectum of single adult human were isolated and cultured. The identification of these cells as stem cells was verified via demonstration of three aspects of their nature: expression of known (a) stem cell markers, (b) self-renewal, and (c) pluripotency.

Expression of appropriate stem cell markers by GI stem cell lines



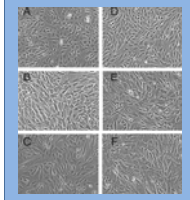
RT-PCR of total RNA prepared from stem cell lines cultured from 10 regions/tissues of the GI tract. All lines were positive for stem cell markers Nanog, LIN28, Oct4 (4th panel down - represents both variant -1 and -2, 5th panel down - variant-2, and 6th panel down - variant-1), and SOX2, though esophagus SOX2 expression was very low. While stem cell lines derived from all tissues demonstrated expression of Bmi1, the duodenum, jejunum, and transverse colon were negative for putative intestinal stem cell marker Lgr5. This may indicate the regional specificity of stem cell marker expression profiles of various segments of the GI tract. Total RNA obtained directly from the same jejunum tissue that the jejunum stem cell line was derived was also negative for Lgr5.

Demonstration of stem cell lines' "self-renewal"

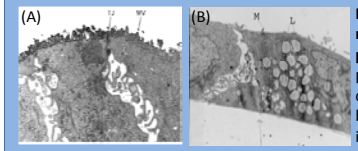


RT-PCR of total RNA prepared from stem cell line developed in our laboratory from a section of jejunum and cultured for 20 passages (10 shown). The jejunum stem cell line continued to replicate and express the stem cell markers Oct4 and Nanog: demonstrating, not only, stability in culture, but also, the ability to maintain its "self-renewal" characteristic during repeated cell divisions in culture. RT-PCR reactions were performed with equal amounts of total RNA and samples were further equilibrated based upon their β-tubulin amplification results.

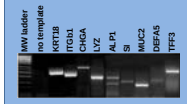
Demonstration of cultured stem cells' pluripotency



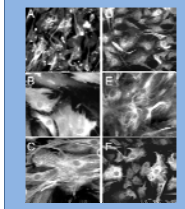
Phase images of representative HIPEC lines derived from various segments of the intestine: (A) duodenum, (B) jejunum, (C) ascending colon, (D) transverse colon, (E) sigmoid, and (F) rectum. All human intestinal primary epithelial cell (HIPEC) lines were derived from stem cell lines established from resected specimens obtained from the normal portions of GI tissue from various patients.



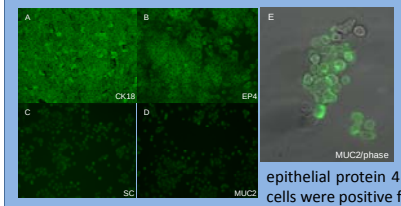
Electron microscopy of a representative HIPEC line at passage 4. (A) Microvilli (MV) are present on the apical surface of cells which contain large nuclei, bundles of microfilaments, and intercellular tight junction (TJ). (B) Goblet cells are also present and are readily identified by their scant microvilli (M) and numerous mucin containing vesicles (L).



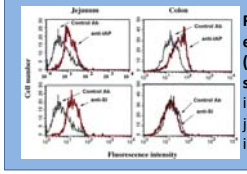
Expression of epithelial markers. RT-PCR products of a total RNA prep from small intestine derived HIPEC cell line A2J1. The cell line demonstrated expression of epithelial markers cytokeratin-18 (KRT18) and β1 integrin (ITGb1), enteroendocrine marker chromogranin A(CHGA) both Paneth markers lysozyme (LYZ) and defensin-5 (DEFA5), both enterocyte markers intestinal alkaline phosphatase (ALP1) and sucrase isomaltase (SI), and both Goblet markers mucin-2 (MUC2) and trefoil factor 3 (TFF3).



Analysis of cytokeratin (CK) in HIPEC lines derived from: (A) duodenum, (B) jejunum, (C) asending colon, (D) transverse colon, (E) sigmoid, and (F) rectum. Dissociated cells from HIPEC monolayers were stained with anti-cytokeratin-18. All cells were positive for cytokeratin.

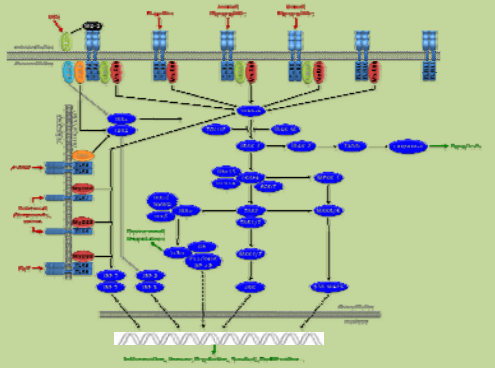


IC staining of HIPEC lines for epithelial lineage specific potein expression. The vast majority of cells were shown to be positive to varying degrees for epithelial markers cytokeratin-18 (CK18) and epithelial protein 4 (EP4). A smaller subset of cells were positive for Goblet cell specific secretory component (SC) and mucin-2 (MUC2).

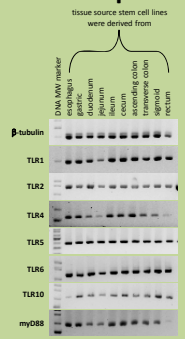


Flow cytometric analysis of epithelia marker expression of HIPEC lines derived from jejunum (A2J1 cell line) and colon (5A cell line) tissue sources. As expected both cell lines express intestinal alkaline phosphatase (IAP) and only the jejunum derived cell line expresses sucrase isomaltase (SI).

Signalling Pathways of Toll Like Receptors (TLRs)

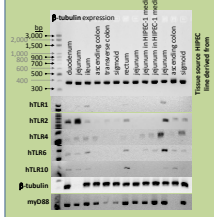


TLR expression pattern of GI stem cell lines



RT-PCR of total RNA prepared from stem cell lines cultured from 10 regions/tissues of the GI tract. All lines demonstrated some variable level expression of TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, and myD88. Rectal derived stem cells showed very low levels of TLR4 and myD88, where as, TLR1, TLR5, and TLR6 expression was fairly consistent in all GI tract derived stem cells lines.

TLR expression pattern of HIPEC lines derived from GI stem cell lines



RT-PCR of total RNA prepared from non-transformed human intestinal primary epithelial cell (HIPEC) lines derived from adult GI stem cell lines cultured from 10 regions/tissues of the GI tract. The gram positive pathogen signaling TLRs (TLR1 and TLR6) were preferentially expressed by small intestinal and the rectal Epi-SCs. Whereas, mRNA for the Gram negative sensory TLR2 and TLR4 was detected in Epi-SCs derived from all segments (small intestine>>large intestine) of the GI tract. Interestingly, TLR10 mRNA expression was strongest in rectum and was higher overall than any other TLR. Additionally, the mRNA level for TLR adaptor protein MyD88 was comparable (with slight variations) in all cell lines. Unlike the stem cell data depicted above, these cells lines were derived from multiple individuals, therefore, the observed variable levels of expression may represent normal variation between individuals. Further whole GI tract analysis will answer this question.

CONCLUSION

Our results suggest a unique expression profile of functional TLRs on epithelial stem cells and epithelial cells derived from stem cells from micro-environmentally distinct regions of the GI tract. These findings will aid in understanding the mechanisms of innate immune response and help to better devise protection against microbial invasion in the intestine and the resulting diseases.