Use of Methylation Patterns to Determine Expansion of Stem Cell Clones in Human Colon Tissue

TREVOR A. GRAHAM,* ADAM HUMPHRIES,* THEODORE SANDERS,*,‡ MANUEL RODRIGUEZ–JUSTO,§ PAUL J. TADROUS,‖ SEAN L. PRESTON,‡ MARCO R. NOVELLI,§ SIMON J. LEEDHAM,*,¶ STUART A. C. McDONALD,*,‡ and NICHOLAS A. WRIGHT*,‡

*Histopathology Laboratory, Cancer Research UK London Research Institute, London; ‡Centre for Digestive Diseases, Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, London; §Department of Histopathology, University College London Hospital, London; ‖Department of Histopathology, Northwick Park Hospital, Harrow, London; and ¶Molecular and Population Genetics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

See editorial on page 1139.

BACKGROUND & AIMS: It is a challenge to determine the dynamics of stem cells within human epithelial tissues such as colonic crypts. By tracking methylation patterns of nonexpressed genes, we have been able to determine how rapidly individual stem cells became dominant within a human colonic crypt. We also analyzed methylation patterns to study clonal expansion of entire crypts via crypt fission. **METHODS:** Colonic mucosa was obtained from 9 patients who received surgery for colorectal cancer. The methylation patterns of Cardiac-specific homeobox, Myoblast determination protein 1, and Biglycan were examined within clonal cell populations, comprising either part of, or multiple adjacent, normal human colonic crypts. Clonality was demonstrated by following cytochrome c oxidase-deficient (CCO^-) cells that shared an identical somatic point mutation in mitochondrial DNA. **RESULTS:** Methylation pattern diversity among CCO^- clones that occupied only part of a crypt was proportional to clone size; this allowed us to determine rates of clonal expansion. Analysis indicated a slow rate of niche succession within the crypt. The 2 arms of bifurcating crypts had distinct methylation patterns, indicating that fission can disrupt epigenetic records of crypt ancestry. Adjacent clonal CCO^- crypts usually had methylation patterns as dissimilar to one another as methylation patterns of 2 unrelated crypts. Mathematical models indicated that stem cell dynamics and epigenetic drift could account for observed dissimilarities in methylation patterns. **CONCLUSIONS: Methylation patterns can be analyzed to determine the rates of recent clonal expansion of stem cells, but determination of clonality over many decades is restricted by epigenetic drift. We developed a technique to follow changes in intestinal stem cell dynamics in human epithelial tissues that might be used to study premalignant disease.**

Keywords: Mitochondrial DNA; Lineage Tracing; Epithelial Cell Dynamics.

CpG rich regions within nonexpressed genes, so called *CpG islands*, exhibit age-related methylation.[1] Methylation at these loci is somatically inherited, and the CpG islands are initially unmethylated in the zygote but acquire de novo methylation stochastically at mitosis. Therefore, comparison of the methylation patterns between 2 cells should reveal their clonal relation: cells with a recent common ancestor should have similar methylation patterns, whereas unrelated cells are unlikely to have similar patterns of methylation (Figure 1). This makes methylation patterns an attractive means to infer the ancestry of cells within the human body, without requiring invasive labelling techniques.

The human intestinal crypt possesses a well-characterized stem cell niche. The crypt population is maintained by a small number of stem cells located at the crypt base.[2] Differentiated cells migrate rapidly from the base and are shed into the lumen. In their seminal work, Yatabe et al used methylation patterns to infer the dynamics of the stem cell population of the crypt. Individual crypts contained a small number of distinct methylation patterns—evidence that the crypt contained multiple long-lived stem cells and, furthermore, that the variation between methylation patterns within a crypt was indicative that the stem cells were competing among themselves to retain a place in the niche.[3,4] Similar niche dynamics have been suggested by analysis of methylation patterns in small intestinal crypts [5] and in other disparate systems such as endometrial glands,[6] hair follicles,[7] and T cells.[8]

These elegant studies essentially exploit 2 properties of nonexpressed gene CpG island methylation. First, that DNA methylation is coupled to the cell cycle, so that tissues maintained by actively dividing progenitor cells slowly accumulate methylation, whereas cells in mitoti-

Abbreviations used in this paper: BGN, Biglycan; CCO, cytochrome c oxidase; *CSX*, Cardiac-specific homeobox; IM, intestinal metaplasia; mtDNA, mitochondrial DNA; *MYOD1*, Myoblast determination protein 1; OAT, O-acetyltransferase; PCR, polymerase chain reaction.

© 2011 by the AGA Institute
0016-5085/$36.00
doi:10.1053/j.gastro.2010.12.036

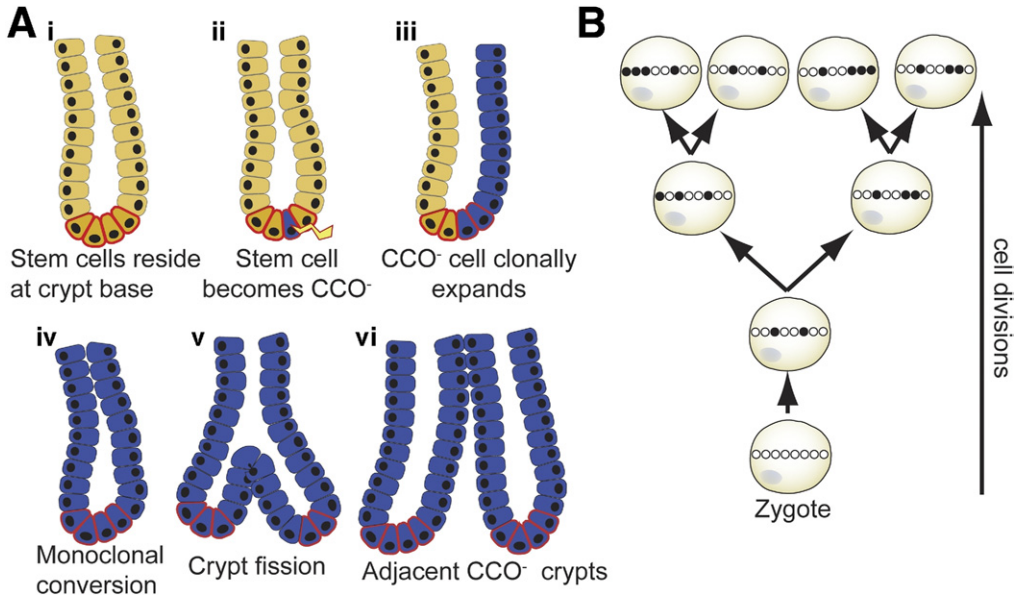

Figure 1. Inferring dynamics of human colon crypts. (*A*) Monoclonal conversion of a crypt. A single stem cell at the crypt base can acquire a CCO deficiency (*i*, *ii*). Clonal expansion of the CCO^- lineage, presumably through random drift, results in monoclonal conversion of the stem cell niche (*iii*, *iv*). A mutated crypt can undergo fission, producing adjacent, clonally related CCO-deficient crypts (*v*, *vi*). (*B*) Methylation patterns record cell ancestry. *Open circles* represent methylated CpG sites; *closed circles* represent unmethylated sites. Certain CpG rich sites nonexpressed are unmethylated in the zygote but become increasingly methylated as a result of stochastic de novo methylation associated with mitosis. Methylation patterns are inherited with reasonable fidelity, so closely related cells are expected to have similar methylation patterns.

cally inactive tissues do not. Therefore, the number of methylated sites within a CpG island, a statistic termed the *percent methylation* of the CpG island, increases with the mitotic age of the tissue.[1] Second, that methylation patterns accumulate stochastically, so that, if 2 cells share similar methylation patterns, it is indicative of their sharing a common ancestor cell. Thus, the variation between methylation patterns shown by a population of cells can be used to infer properties of the stem cell niche, such as stem cell number and the tendency of a stem cell to undergo asymmetric division.[3,4,9] The dynamic nature of the methylation patterns has been at the core of these analyses.

In spite of this dynamism, some studies have attempted to use individual methylation patterns per se to infer clonal relations. Mihara et al found that *ZIK1* promoter methylation patterns differed between individual glands isolated from regions of stomach intestinal metaplasia (IM) in the stomach and so concluded the disease had polyclonal origins.[10] Kim and Shibata compared methylation patterns of nonexpressed genes between individual morphologically normal crypts from the human colon[11] and found that the spatial distance between crypts did not correlate with the *epigenetic distance* between them. Similarly, Siegmund et al investigated the diversity of methylation patterns in individual malignant tubules extracted from colon carcinomas: as in the normal colon, the spatial distance between tubules in the tumor did not correlate with epigenetic distance. The authors concluded that tumor growth was characterized by a rapid initial clonal expansion followed by an extended period of size restriction.[9]

These claims are based on the assumption that methylation patterns record clonality for long periods of time: a property that has never been directly assessed. Here, we investigate the ability of methylation patterns to record clonal relations by comparing methylation patterns between adjacent cytochrome c oxidase (CCO)-deficient (CCO^-) intestinal crypts whose clonality can be demonstrated by the same mitochondrial DNA (mtDNA) mutation. CCO is a mitochondrially encoded enzyme that shows sporadic loss of expression with aging, usually as a result of somatic mtDNA mutation.[12] Through random drift, a CCO^- stem cell can occupy the whole crypt via *niche succession*, leading to a *monoclonally converted*, wholly CCO^-, crypt (Figure 1). Patches of adjacent CCO^- crypts form following *fission* of an ancestral parent crypt because each crypt in the patch has the same mtDNA point mutation.[13] The efficacy of CCO deficiency as a clonal marker has been demonstrated in other epithelial tissues including small intestine,[14] stomach,[15] liver,[16] hair follicle, and pancreas.[17]

Here, the ability of methylation patterns to record clonality is assessed by comparing the methylation patterns between the clonal population of adjacent CCO^- colonic crypts. Methylation patterns of CCO^- clones occupying only part of a crypt are also examined to investigate the dynamics of clonal expansion prior to monoclonal conversion within the stem cell niche.

Download English Version:

https://daneshyari.com/en/article/3294914

Download Persian Version:

https://daneshyari.com/article/3294914

Daneshyari.com