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Differentiation ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Differentiation

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

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ARTICLE INFO

Article history:
Received 13 February 2009
Accepted 7 July 2009

Keywords:
Differentiation
Stem cells
Epigenetics
Intestinal crypts
Methylation
Histone modifications
Embryogenesis
Cancer

ABSTRACT

Establishment and maintenance of epigenetic profiles are essential steps of development during which stem cells, despite identical genetic information, will acquire different and selective gene expression patterns, specific for their fate. This highly complex programming process involves mechanisms that are not yet completely understood although it has been established over the past few years that chromatin modifier enzymes (i.e. DNA and histone methyltransferases, histone deacetylases, histone demethylases, histone acetyltransferases) play essential roles in the establishment of transcriptional programs accompanying cell differentiation. Investigators in this field have been studying a wide variety of cell types including neural, muscular, mesenchymal and blood cells. This review will focus on epithelial cells of the digestive tract, intestinal stem cell niches being a model of choice to understand how epigenetic changes can drive nuclear programming and specific cell differentiation. Moreover, deregulation of epigenetic programming is frequently observed in human tumours and therefore, decoding these molecular mechanisms is essential to better understand both developmental and cancerous processes.

1. Introduction

The two major features of stem cells are their capacity to self-renew for long period through cell division, and to specialize into multiple differentiated cell types under specific biological conditions. Scientists have been using two types of stem cells in their studies to understand mechanisms involved in differentiation and embryonic development. Embryonic stem cells (ESC) are in vitro derivates of the inner cell mass of the blastocyst and retain the ability to differentiate into all cell types of an organism along with unlimited self-renewal capacity. Somatic (or adult) stem cells (SSC) come from many adult organs where they ensure tissue homeostasis. Their renewal and differentiation capacities are limited as they only give rise to all the specialized cell types of the tissue from which they originate.

Both embryonic development and stem cell differentiation in adults involve crucial changes in gene expression profiles, pluripotency factors being silenced while specific genes for one differentiated cell type are activated. This reprogramming is not accompanied by changes in the DNA sequence itself and therefore, it has been established that precise and complex epigenetic rearrangements most likely occur during this process (Hattori and Shiota, 2008).

By definition, the term "epigenetic" refers to a heritable change in the pattern of gene expression that is mediated by mechanisms other than alterations in the primary nucleotide sequence of the gene (Russo et al., 1996). Epigenetic changes in the genome include DNA methylation and histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation), two mechanisms that are often tightly linked in the regulation of gene expression and involved in many cellular processes (Robertson, 2005). For instance, DNA methylation is a crucial epigenetic modification that drives X-chromosome inactivation, genomic imprinting and chromosome stability while histone modifications govern rearrangement of chromatin structure.

DNA methylation occurs on CpG dinucleotides, is mediated by DNA methyltransferases (DNMTs) and follows a cell-specific profile that is established during development and maintained through cell divisions (Wilson et al., 2006). In the human genome, CpG sites are statistically under-represented but are clustered in GC-rich regions termed CpG islands. These CpG islands are often associated with promoter regions or first exons of half the genes in our genome. In normal and differentiated cells, CpG dinucleotides that are not associated with CpG islands (i.e. 80% of them, predominantly in intergenic and intronic regions of DNA, repeat sequences and transposable elements) are heavily methylated whereas CpG islands are usually unmethylated.

DNA methylation by itself does not directly repress transcription. The process of silencing needs the involvement of the epigenetic machinery that controls histone modifications and therefore chromatin compacting. Chromatin modifier enzymes, including histone deacetylases (HDACs) and histone methyltransferases (HMTs), as well as their competing counterpart histone acetyltransferases (HATs) and histone demethylases (Klose and Zhang, 2007; Santos-Rosa and Caldas, 2005; Agger et al., 2008),

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doi:10.1016/j.diff.2009.07.002

are the writers of the histone code, each specific post-transcriptional modification on NH_2-terminal tails of histones being predominantly associated with either gene silencing or activation (Santos-Rosa and Caldas, 2005). In particular, while histone H3 and H4 acetylation is mostly associated with gene expression, di- and trimethylation of H3 lysine 9 (H3K9) and trimethylation of H3K27 elicit the compaction of chromatin and thus gene silencing through the recruitment of heterochromatin protein 1 (HP1) and polycomb group (PcG) proteins, respectively (Bannister et al., 2001; Schlesinger et al., 2007).

Finally, the recent discovery of small non-protein coding RNA named micro-RNAs (miRNAs) that target 3′-untranslated regions (3′-UTR) of genes and either repress messenger RNA maturation or lead to their degradation (Bartel, 2004; He and Hannon, 2004) added a new layer to epigenetic programming of stem cell differentiation.

2. Establishment and reprogramming of epigenetic profiles in early embryonic development

The epigenetic mechanisms involved during the first steps of embryogenesis have been studied for many years and start to be well characterized (Fulka et al., 2008; Heby, 1995).

Within a short period of time after fertilization in mammals, two highly specialized and differentiated parental genomes combine to give rise to an embryo made of totipotent cells with identical genomic characteristics but different fates. Thus, epigenetic rearrangements early after fertilization and during embryogenesis play an evident role in this achievement (Fig. 1). Strongly supporting this statement are initial studies showing the early lethality of embryos in which genes involved in the establishment of epigenetic marks have been knocked out. Indeed, homozygous mouse embryos mutated for Dnmt1 do not survive past mid-gestation (Li et al., 1992), Dnmt3b-deficient mouse embryos show multiple developmental defects after embryonic day 9.5 and are not viable (Okano et al., 1999) while Hdac1-null mouse embryos die at embryonic day 10 due to severe proliferation defects and retardation in development (Lagger et al., 2002). Since then, many studies have shown that extensive epigenetic remodelling indeed takes place early in the development as well as later during terminal cell differentiation (Fig. 1).

First of all, it has been established years ago that oocytes and spermatozoa harbour different epigenetic profiles, the genome of oocytes being globally less methylated than that of spermatozoa (Monk et al., 1987), which is compacted in protamines in place of histones (Balhorn, 2007).

Moreover, while histone modifications of other differentiated cells have been deciphered and clearly associated with either gene silencing or gene activation, it appears that germ cells harbour a very peculiar histone code. In particular, histone acetylation and di- or trimethylation of H3K4 in fully grown germinal vesicule stage oocytes is not necessarily associated with gene expression (Fulka et al., 2008). When the oocyte re-enters meiosis, histones will be rapidly deacetylated, chromatin being condensed and harbouring essentially repressive histone marks.

Soon after fertilization, replacement of protamines from the paternal pronucleus with acetylated histones from the maternal cytoplasm occurs, along with partial to complete demethylation of the paternal DNA. This process implies active demethylation by yet unknown demethylases. The global decondensation of the chromatin is achieved by slower and progressive acetylation of

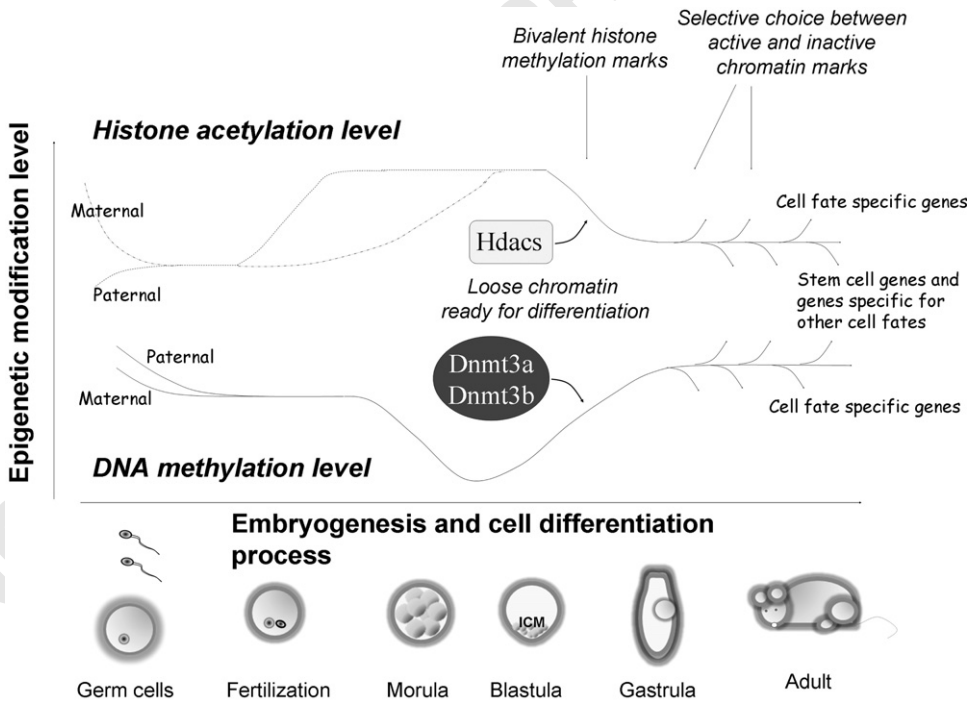

Fig. 1. Epigenetic rearrangements play an essential role in early embryonic development. The oocyte genome is globally less methylated than the spermatozoa genome, which is compacted in protamines, rather than deacetylated histones. Soon after fertilization, these two highly specialized genomes will have to dedifferentiate and subsequently redifferentiate to give rise to all the cell types that compose an adult organism. Protamines that wrap the paternal genome are rapidly replaced by acetylated histones, while the maternal genome undergoes a slower and progressive acetylation. Active and passive demethylation of the fertilized egg will perfect the loose chromatin state acquired as of the morula stage (Heby, 1995). At the blastula stage, the cells of the inner cell mass (ICM) become gradually more methylated compared with the cells of the trophectoderm. After implantation, de novo methylation of the genome is ensured by Dnmt3a and Dnmt3b activity. In the later stages of development, terminal differentiation will be accompanied by precise epigenetic rearrangements allowing expression of cell fate-specific genes while genes involved in proliferation or genes specific from other cell fates will be silenced.

Please cite this article as: Vincent, A., Van Seuningen, I., Epigenetics, stem cells and epithelial cell fate. Differentiation (2009), doi:10.1016/j.diff.2009.07.002

maternal pronucleus (Fig. 1).

Passive, replication-dependent DNA demethylation, rather due to inactivation or sequestration in the cytoplasm of DNMTs, subsequently takes over and continues in Mouse and Human until the morula stage, where the lowest methylation level is observed. Importantly, centromeric heterochromatin, allowing correct cell division and chromosome segregation, as well as imprinted genes, which methylation profile is re-established later according to the sex of the embryo, are protected from active and/or passive demethylation.

After implantation, early expression of Dnmt3a and Dnmt3b allows a progressive de novo remethylation of the embryonic genome that will (i) distinguish between cells from the trophectoderm, which give rise to extraembryonic tissues, and cells from the inner cell mass, which give rise to the embryo itself, and (ii) establish a new methylation pattern specific for pluripotent cells that leaves CpG islands mostly unmethylated (Fig. 1).

3. General characteristics of embryonic stem cells upon self-renewal and differentiation

The epigenetic profile of somatic cells is established later during embryonic development when pluripotent cells specialize to give rise to all types of differentiated cells, specific for each organ. ESCs have been an in vitro model of choice for understanding how these epigenetic mechanisms are established.

Stem cell status involves multiple layers of molecular events designed to impose flexible but precise control over the expression of genes important for development (Lunyak and Rosenfeld, 2008). Several factors of pluripotency and self-renewal have been identified including the Pou domain containing transcription factor OCT4, NANOG, SOX2, FOXD3, REX1, KLF4, c-myc, Lin-28 and the Wnt signalling pathway (O'Shea, 2004; Yeo et al., 2007). Along with expression of these specific factors, stem cells harbour a peculiar epigenetic landscape with a highly dynamic chromatin state characterized by a distinct DNA methylation profile, the genome of ESCs being globally less methylated than differentiated somatic cells (Bibikova et al., 2006). Structural proteins such as HP1 and histone H1 also bind more loosely to chromatin compared with differentiated somatic cells (Chen and Daley, 2008; Collas et al., 2007). These features are essential for chromatin rearrangements and therefore, global transcriptional programming upon differentiation. More particularly, developmentally regulated genes are characterized by the presence of both active and repressive histone marks, where large regions containing trimethylated K27H3 colocalize with smaller regions containing permissive chromatin marks such as methylated K4H3. This chromatin bivalency allows genes encoding specific transcription factors or early differentiation factors to be prepared for rapid transcriptional activation upon differentiation (Bernstein et al., 2006) and, interestingly, is specifically found where responsive elements for previously mentioned pluripotency factors are located. Indeed, crosstalks between these factors and chromatin modifier enzymes occur at several molecular levels. First, these crosstalks include mutual regulation as the promoters of *OCT4* and *NANOG* genes are unmethylated in undifferentiated human ESCs (Lagarkova et al., 2006) and undergo rapid DNA methylation upon differentiation in vitro (Yeo et al., 2007) and after implantation in vivo (Lengner et al., 2007), while OCT4 has been shown to bind to intronic sequences of Jmjd1a gene, encoding histone H3K9 demethylase (Loh et al., 2007). Second, Oct4 and Nanog have recently been shown to interact with each other and associate with Hdac1/2 and Metastasis Associated 1/2, in a newly discovered complex named NODE (for Nanog and Oct4-associated deacetylase), which contained HDAC activity comparable to NuRD complex (Liang et al., 2008). Finally, competition for regulatory elements on key developmental genes between pluripotency factors and chromatin modifier enzymes is indicated by the fact that these factors cannot bind their target genes in differentiated cells due to differences in epigenetic "make-up" of the designated target chromatin (Lunyak and Rosenfeld, 2008).

In stem cells, PcG complex, on the other hand, acts to stabilize a repressive chromatin structure and has been shown to play important roles in the direct silencing of cell differentiation regulators such as Otx2, Satb2 and Tbx3 factors (Pasini et al., 2007) and are part of the preprogrammed memory system established during embryogenesis (Bracken et al., 2006).

Adding to the complexity of epigenetic regulation of stem cell pluripotency, recent studies indicate that miRNAs show distinctive temporal and tissue-specific expression in mammals. MiRNAs are endogenous noncoding RNAs, of 18–24 nucleotides in length, typically excised from 60 to 110 nucleotide foldback RNA precursor structures (Calin and Croce, 2006), which play important negative regulatory roles in animals and plants by targeting messenger RNA transcripts for degradation or translational repression, involving the RNA Interference Silencing Complex (RISC) (Bartel, 2004). Recently, Gonzalez and collaborators showed that miRNAs can also trigger transcriptional silencing via chromatin remodelling (i.e. trimethylation of H3K9) at DNA regions containing complementary sequences (Gonzalez et al., 2008). Many miRNAs are specifically expressed during differentiation and embryogenesis, while ESCs, on the other hand, express a unique set of miRNAs (Houbaviy et al., 2003). Moreover, some miRNAs expressed in stem cells are not matured until differentiation process is initiated such as let-7, which processing is inhibited by a feedback loop involving the pluripotency factor Lin-28 (Rybak et al., 2008), strongly suggesting that miRNAs may have a role in the maintenance of the pluripotent cell state and in the regulation of early mammalian development.

Upon differentiation, a unique DNA methylation profile is acquired, reducing cell pluripotency while inducing progressive activation of cell- and tissue-specific genes (Hattori and Shiota, 2008) (Fig. 2). This activity may be due to Dnmt1, since deficiency in this DNA methyltransferase blocks differentiation potential of ESCs (Jackson et al., 2004). Heterochromatic markers, such as HP1 proteins, change their localization from a hyperdynamic state involving plasticity in chromatin organization, to a more concentrated state, stabilizing the new cell fate. Thus, the chromatin becomes progressively less permissive and loose with a decrease in global levels of active histone marks such as acetylated histones H3 and H4 (Chen and Daley, 2008). Cell differentiation is also accompanied by a specific increase in silencing-associated dimethylated K9H3 and trimethylated K9H3 (Wen et al., 2009), but removal of EZH1/EZH2-mediated K27H3 trimethylation (Shen et al., 2008) presumably by newly discovered histone demethylases (Lunyak and Rosenfeld, 2008).

Finally, bivalent chromatin profiles will be changed either to methylated H3K4 at genes specific for the particular cell fate in which the cells are engaged, or to trimethylated H3K27 at genes specific for other cell fates (Bernstein et al., 2006).

4. Somatic stem cell niches in the intestinal crypts: a model for cell differentiation in adults

The adult small intestine is constructed of millions of crypts and villi lined by a single-layered epithelium, each crypt base containing multiple stem cells that give rise to the four types of differentiated cells in the intestinal epithelium: columnar cells specialized for absorption, enteroendocrine cells for secretion of peptides hormones, goblet cells for mucus secretion, and Paneth

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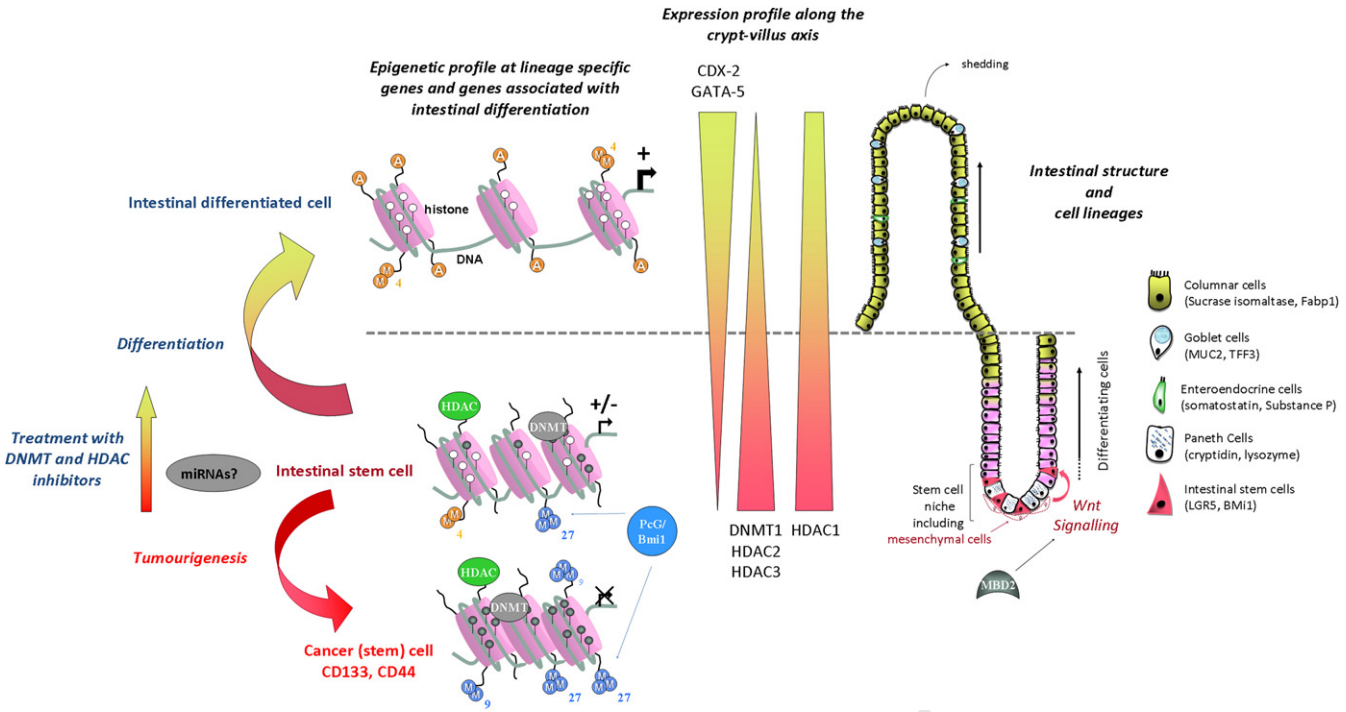

Fig. 2. Model for epigenetic regulation in adult intestinal crypts. The anatomy of the intestinal epithelium, shaped into crypts and villi, is depicted on the right-hand side. Stem cells in red give rise to the four other cell types of the epithelium. Three of them, the absorptive or columnar cells, the goblet cells and the enteroendocrine cells, migrate as they differentiate to the top of the villi. The fourth cell lineage, made of Paneth cells, migrates to the bottom of the crypt. Transit amplifying compartment in pink contains the cells having undergone the process of differentiation. Self-renewal of stem cells is ensured by the Wnt signalling pathway, which is regulated by epigenetic mechanisms involving MBD2. Expression profiles along the crypt–villus axis of GATA-5 and CDX-2 transcription factors, involved in terminal differentiation of intestinal epithelium, as well as expression profiles of HDACs and DNMTS, which may play a role in their silencing at the bottom of the crypt, are shown. On the left-hand side, epigenetic modifications involved in regulation of differentiation genes are presented. Lollipops represent CpG sites, open circles being unmethylated cytosines while black circles being methylated cytosines. Histones are shown as pink cylinders. Specific histone modification profiles are established at each step toward differentiated cells. A: Acetylation, M: Methylation. Numbers refer to the lysine residue on histone H3 which tail is post-transcriptionally modified. Reprogramming of epigenetic modifications leads to dedifferentiation and cancer but is potentially reversible, by the use of HDAC or DNMT inhibitors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells which help keep the crypts sterile (Leedham et al., 2005a) (Fig. 2). The self-renewing epithelium of the small intestine is ordered into stem/progenitor crypt compartments and differentiated villi compartments. The maintenance of undifferentiated proliferative cells in crypts and adenomas requires concerted activation of the Notch and Wnt cascades (Fodde and Brabletz, 2007; van de Wetering et al., 2002; van Es et al., 2005). During epithelial renewal, stem cells migrate out of the niche and up the villus while differentiating and die after a week once they have reached the lumen (Crosnier et al., 2006). Thus, intestinal epithelial cell turnover is particularly fast and occurs from a well-identified structure, so-called niche, which provides an optimal microenvironment for stem cells, ensured by epithelial cells, mesenchymal cells and extracellular substrates (Scoville et al., 2008; Walker and Stappenbeck, 2008). Therefore, while ESCs, although a reasonably good model in vitro, harbour epigenetic variations and reprogramming during extended culture (Collas et al., 2007), the intestinal epithelium represents an attractive biological model of differentiation in which most of the previously cited pluripotency and differentiation signalling pathways are involved (Yen and Wright, 2006), even though some differences have been reported. For instance, while Oct4, a well-established regulator of pluripotency in the inner cell mass of the mammalian blastocyst as well as in embryonic stem cells, is detected in a variety of SSC and tumour cells, its activity is dispensable for both self-renewal and maintenance of SSCs in the adult mammal (Lengner et al., 2007).

Over the past years, isolation and growth in culture of intestinal crypts has challenged researchers who have developed elegant methods to be able to decipher mechanisms underlying intestinal SSC differentiation (Cano-Gauci et al., 1993; Tou et al., 2004; Grossmann et al., 1998). Indeed, although the structure of the crypt itself is well characterized and strong evidence of the presence of stem cells in this compartment has been established several decades ago (Marshman et al., 2002), the definitive identification of the proper stem cells lacks of unique molecular markers. Thus, the exact number (rather 4 or 6 stem cells per crypt), location (either at position +4 relative to the crypt bottom, or hidden between Paneth cells and identified as crypt base columnar cells), and growth rate of intestinal stem cells is still unknown with certainty (Barker and Clevers, 2007).

The RNA binding protein Musashi-1, which expression in neonatal, adult, and regenerating mouse crypts is consistent with the predicted number and distribution of intestinal stem cells, has been proposed as a marker for stemness (Potten et al., 2003). However, among the potential stem cell markers, genes targeted by the Wnt signalling pathway may represent some of the best candidates. Indeed the Wnt/β-catenin/T-Cell factor-lymphoid enhancing factor signalling pathway is the most dominant force in controlling cell fate along the crypt–villus axis and has been shown to specifically target the stem cell niche at the bottom of intestinal crypts, where the stem cells are located (Pinto and Clevers, 2005; Wong et al., 2002) (Fig. 2). Thus, Clevers and collaborators have conducted numerous studies to track down the stem cells of the intestine (Barker and Clevers, 2007). Recently,

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they identified Lgr5, an orphan-protein coupled receptor as a marker of stem cells in mouse small intestine (Barker et al., 2007) sustaining the hypothesis of crypt base columnar cells as being the true stem cells.

As previously mentioned, several layers of molecular complexity drive the differentiation of stem cells. The signalling pathways and the numerous transcription factors involved in the elaboration of the four different cell lineages present in the intestine, and therefore in gut homeostasis, are now better understood and have recently been reviewed (Yen and Wright, 2006; de Santa Barbara et al., 2003; Radtke and Clevers, 2005; Leedham et al., 2005a; Buisine et al., 2008). The epigenetic rearrangements underlying expression of these differentiation factors, on the other hand, still need to be better characterized (Vincent and van Seuningen, 2008).

For this purpose the *Drosophila* seems to be an adequate animal model. A very recent study showed that *Drosophila* gene, *scrawny*, encoding a ubiquitin-specific protease, is required in intestinal stem cells, functions in gene silencing to deubiquitylate histone H2B, and represents a common mechanism within stem cells that is used to repress the premature expression of key differentiation genes, including Notch target genes (Buszczak et al., 2009).

On the other hand, the development of a reliable ex vivo model for mouse intestine differentiation by Tou and collaborators allowed these authors to investigate the functions of class I HDACs in mammalian intestine development and cytodifferentiation (Tou et al., 2004). In particular, they showed that HDAC1 and HDAC2 expression level decreased significantly upon tissue differentiation. HDAC3 (Wilson et al., 2006) and DNMT1 (De Marzo et al., 1999) harbour the same expression profile along the crypt–villus axis (Fig. 2). From this, one can speculate that activation of maturation-associated genes encoding intestine-specific markers is driven by acetylation of core histone proteins and demethylation at their promoters. Confirming this hypothesis, a recent study in rats showed that sucrose isomaltase, a marker of terminal differentiation of absorptive cells, was induced during the transition from the crypt to the villi by a switch in histone modifications at the promoter region from di- and trimethylated H3K9 to diacetylated H3K9/K14 (Suzuki et al., 2008) (Fig. 2).

Accordingly, numerous studies have shown the positive effect of Class I HDAC inhibition on expression of terminal differentiation markers in the intestine by using HDAC inhibitors (Fig. 2). Valproic acid has been shown to accelerate cytodifferentiation and activate the expression of intestinal markers such as apolipoprotein 1A, intestinal and liver fatty acid-binding proteins 1 and 2 (Tou et al., 2004), while sodium butyrate specifically induces the expression of absorptive cell markers, such as the mucin MUC3 and alkaline phosphatase (Velcich et al., 1995), to the detriment of secretory markers, such as trefoil factor 3 (TFF3) (Augenlicht et al., 2003). Our recent work showed that the effect of sodium butyrate on expression of the goblet cell marker mucin MUC2 is dose-dependent and is associated with increased acetylation of histones H3 and H4 and methylation of K4H3 at the promoter (Burger-van Paassen et al., 2009). Finally, trichostatin A increases significantly the expression of MUC2 in undifferentiated colonic cells (Vincent et al., 2007). HDAC3 gene silencing, on the other hand, induces expression of alkaline phosphatase in colon cancer cell lines (Wilson et al., 2006).

Furthermore, we recently demonstrated that MUC2 is silenced by DNA hypermethylation in colonic undifferentiated cells that undergo demethylation of MUC2 promoter once they reach confluence in culture and start differentiate. This finding led us hypothesise that epigenetic silencing of MUC2 would be a mechanism used by intestinal stem cells not yet engaged in the differentiation process to maintain their undifferentiated state (Vincent et al., 2007). Similarly, TFF3 gene has been shown to be repressed by methylation in organs that do not express this intestinal goblet cell marker (Ribieras et al., 2001), while cell differentiation-dependent epigenetic modifications at the 5′-UTR of the gene encoding the mucin MUC4, a differentiation marker of intestinal cells (Jonckheere et al., 2007), have been shown to occur in epithelial cancer cells (Vincent et al., 2008).

These studies confirm that epigenetic reprogramming occurs at genes encoding specific markers for the four types of mature cells in the gut, allowing their activation upon maturation of intestinal stem cells. Thus, chromatin modifier enzymes, may also represent good candidate markers for the identification of intestinal stem cells. Interestingly, Bmi1, a member of the PcG gene family part of the PcG-repressing Complex 1 already known to be involved in the self-renewal of neuronal, hematopoietic and leukemic cells, has been recently identified as an intestinal stem cell marker (Sangiorgi and Capecchi, 2008).

Other groups have shown that regulation of key developmental pathways is also ensured by chromatin modifier enzymes. In particular, the methylcytosine binding domain 2 (mbd2) regulates Wnt signalling and binds the Lect2 promoter in association with NuRD (Phesse et al., 2008) (Fig. 2).

Conversely, while isolation of human intestinal SSCs is precluded, analysis of methylation patterns in human colonic or intestinal crypts has proven to help reveal niche characteristics (Kim et al., 2005b; Kim and Shibata, 2002; Nicolas et al., 2007). Search for methylation tags has also been a strategy to better determine stem cell number and how they divide in the crypt (Yatabe et al., 2001). The elegant tracing strategy adopted in these studies confirmed that (i) all epithelial cell types derive from a single stem cell, (ii) that crypts contain multiple stem cells and can multiply through lateral fission and (iii) that age-dependent methylation profiles are established in the intestinal crypts.

Thus, models and techniques used to isolate the intestinal crypts at different heights along the crypt–villus axis (Suzuki et al., 2008) and the recent identification of intestinal stem cell markers (Barker et al., 2007; Sangiorgi and Capecchi, 2008), coupled with new high throughput techniques aimed at studying epigenetic landscapes (methylated CpG island amplification (MCA), followed by hybridization on microarrays (Omura et al., 2008), methylated DNA immunoprecipitation (MeDIP)-on-chip (Keshet et al., 2006), or ultradeep bisulfite sequencing (Taylor et al., 2007)) will certainly bring new insights into the mechanisms of embryonic development and cell differentiation (Fig. 2).

Regarding miRNAs in intestinal stem cells, not much is known but overlapping miRNA profiles between normal intestinal cell and colon cancer cells indicated that miRNA mi-R-17-92 and its target E2F1 may play a role in colonic crypt cell transformation into cancer cell. Enrichment of miRNA mi-R-17-5p was also found in the proliferating crypt compartment (Monzo et al., 2008). Some miRNAs are also induced by transcription factors responsible for intestinal cell differentiation (Hino et al., 2008). Much more work is surely needed to identify pattern of miRNA expression and their involvement in neoplastic transformation of the colonic epithelium.

5. Abnormal epigenetic reprogramming, carcinogenesis and cancer stem cells

Because ESCs share certain characteristics with cancer cells (lack of senescence, regulation of cell cycle, and contact inhibition) and altered patterns of epigenetic modifications observed in cancer cells are sometimes reminiscent from early embryonic development, deciphering the mechanisms of establishment of epigenetic marks in the genome could help understand the

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process of dedifferentiation undergone by normal cells during early steps of carcinogenesis (Fig. 2).

Indeed, repressive chromatin marks associated with the promoters of silenced genes in cancer cells resemble the chromatin modifications inherent to those same genes in normal stem cells. In particular, bivalent chromatin marks specific for ESCs are often found at genes epigenetically silenced in cancer cells and may predispose these tumour suppressor genes to hypermethylation (Ohm et al., 2007).

Methylation and histone modification profiles of SSCs, however, are significantly different from ESCs, differentiated cells and from cancer cells (Bibikova et al., 2006), the latest being characterized by an added layer of complexity (Ohm et al., 2007). Although only a small number of CpGs contributes to this difference, the methylation pattern of a small number of developmentally controlled genes may constitute a useful epigenetic mark, unique for each type of cells, and may help distinguish between normal and cancer cells in the early events of carcinogenesis. Moreover, it suggests that if stem cells do not respond properly to the differentiation signal and do not direct the appropriate epigenetic rearrangements, they may proliferate wildly, resulting in cancer. This statement has been extensively studied at the genetic and transcriptional level in the gut, where it is thought that uncontrolled stem cells are the initiators of gastrointestinal cancers (Leedham et al., 2005b; Barker et al., 2009), and has been recently confirmed as Lgr5 expression pattern, the recently highlighted marker of intestinal stem cells, in mouse and human adenomas resembles its expression pattern in the normal intestinal crypts (Becker et al., 2008; Barker et al., 2009). Similarly, over-expression of Bmi1, the other intestinal SSC marker, has been correlated with the malignant grades of human digestive precancerous tissues, which suggests that advanced Bmi1 dysregulation might predict malignant progression (Tateishi et al., 2006).

The hypothesis of crypt stem cells being the cells-of-origin of intestinal cancer is further supported by epigenetic evidence. In particular, misexpression of numerous Wnt signalling pathway antagonists has been attributed to changes in the methylation profile of the genes. For instance, silencing of adenomatous polyposis coli (APC) gene has been found in colorectal cancers (van der Flier and Clevers, 2008) and has been associated with promoter hypermethylation (Esteller et al., 2000), even though APC promoter hypermethylation alone does not seem to result in complete gene inactivation (Segditsas et al., 2008). Similarly, secreted frizzled-related proteins (SFRPs), identified and reported to act as inhibitors of the Wnt signalling pathway, are hypermethylated in microsatellite unstable colorectal cancers (Tanaka et al., 2008). Moreover, methylation of APC1A, APC2, SFRP1, and SFRP2 appears to mark progression from colitis inflammatory bowel disease to inflammatory bowel disease-associated neoplasia, and these genes may serve as biomarkers for this disease (Dhir et al., 2008). SOX7, a suppressor of β-catenin-mediated transcriptional activity was also found to be silenced by methylation in human colorectal cancer cell lines (Zhang et al., 2008). Along with changes in methylation profiles, members of the Wnt signalling pathway undergo dramatic changes in chromatin marks in colon cancer. HDAC3 over-expression in half of colon adenocarcinomas favours β-catenin translocation to the nucleus and inhibits three known Wnt inhibitors, namely TLE1, TLE4 and SMO (Godman et al., 2008).

Along with the Wnt signalling pathway, the bone morphogenic proteins (BMPs), members of the TGFβ growth factor superfamily, have well-described roles in the crypt–villus axis development where BMP-4 functions as a negative regulator of intestinal stem cell proliferation (Yen and Wright, 2006). Disrupted BMP signalling and a role for BMP3 in colorectal cancer in tumour development has recently been investigated. Indeed, BMP3 has been found to be down-regulated in colorectal cancer by extensive hypermethylation correlated with microsatellite instability and the CpG island methylator (Loh et al., 2008).

In addition, cancer cells undergo epigenetic changes throughout the differentiation pathway, inducing the silencing of markers of terminal differentiation as well as transcription factors involved in their expression. In particular, p16 (Lee et al., 2006), CDX-2 (Kawai et al., 2005) or GATA-4/-5 (Akiyama et al., 2003) transcription factors are silenced by promoter hypermethylation or by PcG proteins in colorectal cancer (Visvader and Lindeman, 2008). We and others have shown that MUC2 gene, which encodes a secreted mucin and intestinal goblet cell marker, is silenced by hypermethylation and histone deacetylation in colorectal cancers (Vincent et al., 2007; Gratchev et al., 2001). By genome-wide search, other genes encoding terminal differentiation markers, such as the enteroendocrine cell markers somatostatin and Tachykinin-1, were also found to be epigenetically silenced in colon cancer (Mori et al., 2006). Thus, this epigenetic mechanism locks the entire signalling cascade responsible for differentiation, ensuring that cancer cells maintain their undifferentiated state and their proliferative skills.

Tumours harbour stem cell population markers, such as CD133 (encoded by the PROM1 gene), CD44 or EpCAM (epithelial cell adhesion molecule) in colorectal cancer (Visvader and Lindeman, 2008). They have heterogeneous cell population among which only cell subsets have the capacity to regenerate a tumour, and are driven by the upregulation of pathways essential for maintenance of stem cells, such as the Wnt/β-catenin pathway (Humphries and Wright, 2008), while genes involved in cell differentiation, are silenced by promoter hypermethylation or by PcG proteins. Thus, it has been suggested that stem cells or early progenitor cells may be the precursors of "cancer stem cells" (Visvader and Lindeman, 2008). The discussion about the existence and significance of the cancer stem cell, opposing to the clonal evolution model of a tumour, has been going on since the concept was proposed. Cancer stem cells are supposed to be the root of tumour recurrence and metastasis, i.e. cells within a tumour that possess the capacity to reform tumours with all its heterogeneity (Fodde, 2009). However, cancer stem cells are difficult to isolate and therefore still have to be characterized, especially in solid tumours, where specific markers still need to be found. For these reasons, understanding the epigenetic mechanisms driving (de)differentiation of stem cells is crucial. In this regard, it has been proposed that investigating miRNAs, expressed as specific sets in normal stem cells and playing an important role in maintenance of their self-renewal capacity, would be of great interest for elucidating characteristics of cancer stem cells (Xia, 2008).

6. Concluding remarks

Although most of embryogenesis studies have focused on genetic and transcriptional regulation, epigenetic programming is a crucial step during development and differentiation in mammals and deserves particular attention in a way to better understand the mechanisms underlying occurrence of severe diseases, including cancer. While serious ethical issues are related to the use of human ESCs, researchers have developed parades and alternative methods to be able to study differentiation mechanisms. Among them, nuclear transfer, altered nuclear transfer, and parthenogenesis bring their own set of advantages and disadvantages (Kastenberg and Odorico, 2008). This review focused on adult intestinal niches as a model of choice in which stem/progenitor cells are localized in the crypts, a well-identified structure that can be isolated for various types of studies in vitro,

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in vivo and in silico. Indeed, mathematical models including cell proliferation, migration, differentiation, crypt fission, genetic instability, APC inactivation and tumour heterogeneity may also help biologists better understand stem cell dynamics (Ro and Rannala, 2001) and how stem cells of the crypt system and colon carcinogenesis function and derive from each other (van Leeuwen et al., 2006; Johnston et al., 2007).

Genetic and epigenetic reprogramming of signals involved in development and differentiation frequently occurs in cancer and has major implication for diagnosis, prognosis, and treatment. Indeed, the pathways that are involved in cell differentiation are commonly impaired, especially in gastrointestinal cancers (Fodde, 2009; Bach et al., 2000). The very first step of carcinogenesis still has to be identified with certainty but some clues have been brought by recent studies showing age-related methylation and chromatin remodelling in small intestine (Kim et al., 2005b; Kirkwood, 2004; Martin et al., 1998). Moreover, epigenetic profiles including miRNAs, which already helped identify stem cells, will surely lift the veil concerning the controversial concept of cancer stem cells.

The discovery of epigenetic differentiation programming of in cancer gave rise to new treatment strategies, as this mechanism of gene silencing is reversible. Indeed, pharmacological inhibitors of class I and II HDAC activity have been identified as potent inducers of growth arrest, differentiation and apoptosis of colon cancer cells in vitro and in vivo (Mariadason, 2008). Interestingly, epigenetic remodelling, as a precursor event of gastrointestinal cancers, raises the possibility of cancer prevention by an adequate diet. In particular, dietary HDAC inhibitors have been identified in cruciferous vegetables, such as broccoli (Dashwood and Ho, 2007). However, neo-adjuvant therapy in cancer does not seem to be able to modify crypt methylation pattern, which suggests that stem cells are relatively protected from transient environment changes (Kim et al., 2005a).

Inflammatory bowel diseases such as ulcerative colitis and Crohn's disease as a risk to develop colorectal cancer for the patient occurs in 5% of the cases. A recent study showed that methylation may link both situations with inflammatory gut leading to a new aberrant DNA methylation signature also observed later in carcinogenesis (Hahn et al., 2008). Interestingly this mechanism is mediated by PcG complex, known to play a crucial role in cell differentiation during development. Modifications of the methylation pattern may also be involved in polyclonal disorders as a switch between preneoplastic stages and progression toward carcinoma. It was recently shown that in non-inherited polyclonal disorders such as gastric cancers associated with intestinal metaplasia an independent aberrant methylation of multiple genes characterized metaplastic glands that later may become cancerous (Mihara et al., 2006).

Finally, epigenetically induced pluripotency may help stem cell research for the elaboration of new hopeful treatments for various neurodegenerative diseases such as Parkinson's disease (Xi and Zhang, 2008), amyotrophic lateral sclerosis, Alzheimer's disease or Huntington's disease (Hou and Hong, 2008), as well as autoimmune diseases such as multiple sclerosis (Mancardi and Saccardi, 2008), spinal cord injuries (Amoh et al., 2008), and muscle damage (Sharma and Raghubir, 2007) by cell transplantation therapies.

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Please cite this article as: Vincent, A., Van Seuningen, I., Epigenetics, stem cells and epithelial cell fate. Differentiation (2009), doi:10.1016/j.diff.2009.07.002