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Review

The role of gene mutations and gene products in intestinal tissue reactions from ionising radiation

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ABSTRACT

The response of the intestine to (low linear-energy-transfer) ionising radiation is reviewed regarding the cellular basis to the reactions, the regenerative processes which restore the tissue, and external agents which aid its recovery. In the steady-state, it is generally considered that the crypt cell lineages in both small and large intestine are maintained by a small number of stem cells, but there are differences for example in the composition of their niche residence and in the numbers of transit cell generations. Various cell surface markers are now available to indentify particular lineage cell types. Radiation doses up to 1 Gy cause apoptotic stem-cell death in particular locations, at higher doses to >6 Gy $Lgr5^+$ stem cells are required for normal intestinal recovery, and at >8 Gy some crypts are sterilised and the probability of animal death from intestinal injury increases with higher doses. Mutations in repair genes, tumour suppressor genes, and survival genes cause various degrees of stem cell and clonogenic cell radiosensitisation. Recent evidence is suggesting much plasticity in the crypt cell lineage, potentially contributing to flexibility in the hierarchical lineage, clonogen number variations and the sensitisation differences. Knockout mice for many different genes have been used to detect their role in both steady state and in irradiated conditions, expected to lead to further insight to the damage and restorative processes. Many different external agents have been used to ameliorate intestinal reactions, including prostaglandins, interleukins, angiogenic and epithelial growth factors, other cytokines, and intraluminal factors.

1. Introduction

The intestinal mucosa is one of several renewing tissues that respond rapidly after irradiation. Although there is some cell death detected even after doses less than 1 Gy, there is a threshold dose for crypt sterilisation of about 8 Gy and for animal lethality from intestinal injury of about 12 Gy [1]. The intestinal architecture and the sequence of injury and recovery events after irradiation are well known in different species. In recent decades there has been much investigation of the cell lineages in the mucosa, the cellular and tissue mechanisms involved in the intestinal radiation syndrome (often called the gastrointestinal or GI syndrome), and agents which alleviate the injuries. In the last few years the cell lineage studies have intensified, using molecular biomarkers to

Abbreviations: Ascl2, achaete-scute family bHLH transcription factor 2; ASM, ase acid sphingomyelinase; ATM, ataxia telangiectasia mutated; Bak1, Bcl2 antagonist/killer 1; Bax, Bcl2 associated X; Bcl-2, B-cell lymphoma 2; bFGF, basic fibroblast growth factor; Bmi1, B lymphoma Mo-MLV insertion region 1 homolog; BMP, bone morphogenetic protein; BMT, bone marrow transplants; CBC, crypt base columnar; DLL1, delta-like 1; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; EGF, epidermal growth factor; FGF, fibrobrast growth factor; Fzd, Frizzled; GH, growth hormone; GLP-2, glucagon-like peptide-2; GSK-3, glycogen synthase kinase 3; Hopx, homeodomain-only protein; ICRP, International Commission on Radiological Protection; IGF-1, insulin-like growth factor 1; KGF-1, keratinocyte growth factor; KLF4, Krüppel-like factor 4; KLF5, Krüppel-like factor 5; Krt19, keratin-19; Lgr5, leucin-rich repeat-containing G protein-coupled receptor 5; LI, labelling index; Lrig1, leucine rich repeats and immunoglobulin like domains 1; MIP-1a, macrophage inflammatory protein 1 alpha; mTert, mouse telomerase reverse-transcriptase; MTG16, myeloid translocation gene 16; Olfm4, olfactomedin 4; PAI-1, plasminogen activator inhibitor type 1; PARP-1, poly (ADP-ribose) polymerase 1; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; PUMA, p53-upregulated modulator of apoptosis; rhEGF, recombinant human epidermal growth factor; SCF, stem cell factor; scid, severe combined immunodeficiency; Snai1, snail family transcriptional repressor 1; Sox9, sex determining region Y box 9; STAT5, signal transducer and activator of transcription 5; TGFb3, transforming growth factor beta 3; TGIF1, TG-interacting factor 1; TLR3, Toll-like receptor 3; VEGF, vascular endothelial growth factor.

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more accurately identify stem cells of different types, and mouse mutants to identify the role of particular genes in the radiation responses. These aspects are reviewed in the present article.

2. Tissue structure of small and large intestine

The tissue structure of the small and large intestine has been described comprehensively [1,2]. Around birth, epithelial proliferation is confined to shallow pockets residing between the villi of the small intestine of mice. Mature small intestinal crypts appear in the first few weeks after birth, by a process in which the intervillus pockets invade the wall of the small intestine. Similarly, colonic crypts become progressively deeper in early postnatal life. Intervillus pockets are initially polyclonal, but rapidly become monoclonal [3]. The process of the monoclonal conversion is considered to be induced by neutral drift in the stem cell pool under steady-state conditions [4]. In order to accommodate the growth of the organ into adulthood, the number of crypt units steadily increases by crypt fission, a process in which new crypts form by branching off existing crypts [5].

It has been estimated that there are about 5×10^7 crypts in the small intestine in man [1]. The length of the human small intestine is about 270 cm, the diameter is about 2 cm, the surface area is about 1620 cm^2, and hence the crypt density is about 3×10^4 per cm^2. Several stem cell types have been described in crypts of the small intestine (see below). Crypts in the colon are larger than in the small intestine, so the density may be around 2×10^4 per cm^2 or even less. The human colon is about 110 cm long, the diameter is about 5 cm, and the surface area is about 1650 cm^2 which is similar to that of the small intestine. Hence, likely there are $\leq 3 \times 10^7$ crypts in the large intestine in man. In a mouse colonic crypt, one estimate is that there are about 6 (to within a factor of 2–3) stem cells, and probably the same in man [6]. Hence, there may be a total colonic stem cell population in man of $\leq 2 \times 10^8$. This is a very rough estimate with large uncertainties.

3. Cell population lineages, kinetics, niche, and signals

As the mammalian gastrointestinal (GI) tract develops from the embryonic gut, it is made up of an endodermally-derived epithelium surrounded by cells of mesodermal origin. Cell signalling between these two tissue layers plays a critical role in coordinating patterning and organogenesis of the gut and its derivatives. Many lines of evidence have revealed that Wnt signalling is the most dominant force in controlling cell proliferation, differentiation and apoptosis along the crypt-villus axis. Wnt mRNA expression in intestinal subepithelial myofibroblasts and Frizzled (Fzd) mRNA expression has been found in both myofibroblasts and crypt epithelium, as part of the stem cell "niche" [7]. Moreover, there are many other factors, for example, bone morphogenetic protein (BMP), 'homeobox', 'forkhead', 'hedgehog', 'homeodomain', and platelet-derived-growth-factor (PDGF) that are also important to stem cell signalling in the intestinal tract.

Although dependent on the tissue type, the adult stem cell niche is usually established around the perinatal to postnatal period. For example, the intestinal tract in the mouse is formed as a simple tube of epithelial cells with high proliferative activity. The first differentiation of these equipotential epithelial cells, or fetal stem cells, is the formation of the villi at embryonic day 15, which are necessary for absorption of nutrition after birth. The formation of crypts that provide an adult-type stem-cell niche for the maintenance of tissue proliferation appears only at postnatal day 7 [8]. The neonatal crypt is occupied by a polyclonal population of stem cells, and monoclonality is established at about 2 weeks after birth [3]. This monoclonal conversion demonstrates the occurrence of competition among fetal stem cells for occupancy of the niche.

Recent advances of stem cell research have demonstrated a scheme of stem cells in crypts of the small and large intestines in mice (Fig. 1).

There are three distinct stem cell populations located at the 4th position from the crypt base (P4) in the small intestine, which are highly apoptosis-sensitive P4 stem cells, as well as more radioresistant and quiescent B lymphoma Mo-MLV insertion region 1 homolog (*Bmi1*)-marked stem cells, also marked by homeodomain-only protein (*Hopx*) [9] and leucine-rich repeats and immunoglobulin-like domains protein 1 (*Lrig1*) [10]. In addition, there are rare P4 stem cells with mouse telomerase reverse-transcriptase (*mTert*) expression [11]. There are also some rapidly-cycling columnar stem cells at the crypt base, which are positive for leucine-rich repeat-containing G protein-coupled receptor 5 (*Lgr5*) and less sensitive to apoptosis [12]. Estimates are 5–14 crypt base columnar (CBC) stem cells per crypt [13,14]. Other markers of these stem cells also have emerged [15], including olfactomedin 4 (*Olfm4*) and achaete-scute family bHLH transcription factor 2 (*Ascl2*) [16], and sex determining region Y box 9 (*Sox9*) [17,18]. $Lgr5^+$ cells contribute to homeostatic cell renewal, whereas $Bmi1^+$ cells proliferate after irradiation to clonally repopulate multiple crypts [19]. $Lgr5^+$ stem cells in the colon were more radiosensitive

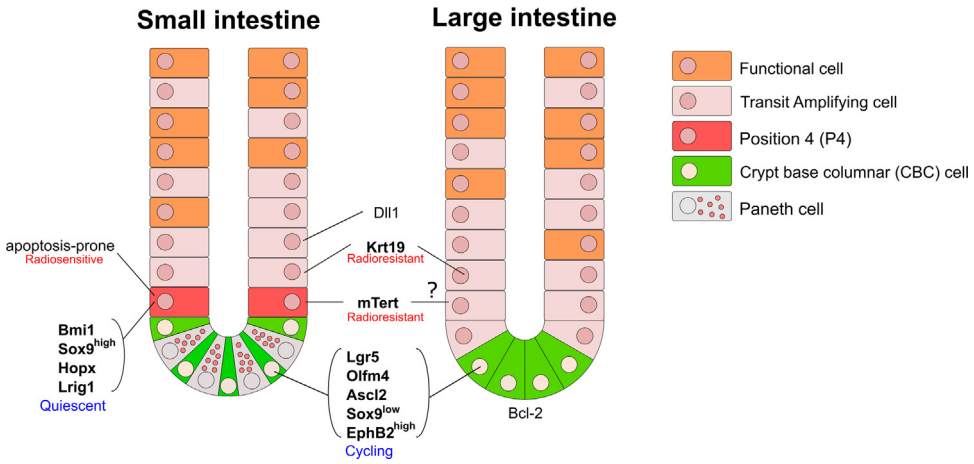

Fig. 1. Stem cell populations in small and large intestinal crypts in adult mice. Bold gene name denotes common stem cell markers. Markers for quiescent stem cells located at position 4 from the bottom of the crypt in small intestine. Functional cells include enteroendocrine cells, goblet cells, enterocytes.