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Digesting the Importance of Cell Fusion in the Intestine

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See rebuttal on page 303.
See counterpoint on page 304.

The implications of cellular fusion on human life stretch far beyond the union of sperm and egg, playing a critical role in cancer pathogenesis and contributing to the regenerative potential of the intestine. Cellular fusion, either homotypic (between identical cells) or heterotypic (between distinct lineages), generates hybrid cells containing variable combinations of parent cell cytoplasm and nuclear material, which ultimately influences cellular identity and function. Although well accepted in developmental biology, the relevance of heterotypic fusion in maintaining homeostasis and in pathology is underappreciated. We assert the importance of cell fusion at the intersection of inflammation, injury repair, and malignancy in the gastrointestinal tract, where fusion gives rise to diverse cell populations and tissue heterogeneity (Figure 1*A*).

Cell Fusion Imparts Cellular Heterogeneity and Functionally Contributes to Tumor Progression

Studies in murine models (Figure 1*B–D*) and human cancer patients (Figure 1*E* and *F*) highlight the functional contributions of cell fusion to tumorigenesis and the metastatic cascade (Figure 1). In mice, we identified fusion between donor bone marrow–derived cells (BMDCs) and recipient premalignant intestinal epithelium (Figure 1*B*) using bone marrow transplant (BMT) and confocal microscopy.[1] Furthermore, non–protein-based paradigms identified genetic alterations in hybrids via Y-chromosome detection in premalignant epithelia of female $Apc^{Min/+}$ mice receiving a male BMDC transplant.[1] Similarly, genetic mixing in fusion was shown after transplantation of BMDCs expressing intestinal-specific Cre recombinase into $Apc^{fl/fl}$ mice, which led to the development of intestinal adenomas.[2] The genetic mixing from fusion also can result in heterogeneous changes in chromosomal structure and copy number, which are critical steps in the formation of many cancers.[3] This evidence indicates an important yet incompletely understood role of fusion in the progression of premalignant states through nuclear reprogramming and genetic alteration.

Macrophages play an undisputed role in inflammatory states, including within the tumor microenvironment and in response to pathogens, where macrophages can fuse into multinucleated giant cells aiding host defense. Readily capable of both homotypic and heterotypic fusion, it is intuitive that the macrophage is a primary fusion partner in inflammatory states,[1] which has paradoxic deleterious implications in malignancy. Because macrophage-neoplastic cell fusion results in phenotypic and genotypic reprogramming, resultant fusion hybrids show characteristics of both parental lineages, which they maintain through subsequent divisions. We captured spontaneous fusion between red fluorescent protein (RFP)-expressing murine colon cancer cells ($MC38^{H2BmRFP}$) and green fluorescent protein (GFP)–expressing macrophages using in vitro live imaging; importantly, GFP^+/RFP^+ fusion hybrids were capable of mitotic division into daughter cells with retained fusion phenotypes.[1,4] Similarly, in vivo–derived MC38 fusion hybrids maintained both parental phenotypes and contributed to tumorigenicity (Figure 1*C*), consistent with our published studies on murine melanoma-derived hybrids.[4] In-depth analyses of in vitro– and in vivo–derived hybrids showed that cell fusion imparts macrophage-associated behaviors onto neoplastic cells, including enhanced chemotaxis, extracellular matrix invasion, and tumorigenic growth in both primary and metastatic sites compared with unfused cancer cells.[4] In addition, in human recipients of sex-mismatched BMT, we leveraged the Y-chromosome to identify fusion-derived neoplastic cells in both primary gastrointestinal tumors (Figure 1*E*) and in circulation.[4] Termed *circulating hybrid cells*, fusion hybrids are detectable in the peripheral blood of patients with gastrointestinal malignancies by the co-expression of epithelial and leukocyte antigens (Figure 1*F*). Circulating hybrid cells correlated with stage and survival in pancreatic cancer patients,[4] highlighting their translational potential as a novel biomarker.

Cell Fusion Complements Stem Cell–Mediated Epithelial Regeneration

Whether in the setting of neoplasia or injury, inflammation plays the role of matchmaker in macrophage heterotypic cell fusion, which ultimately results in genome mixing, diverse cell clones, and tissue heterogeneity. Models of intestinal inflammation (interleukin 10 double-knockout and chemically induced colitis) showed augmented epithelial cell fusion, which was reduced by anti-inflammatory agents.[2] The intestinal stem/progenitor cell is likely the primary fusion partner of BMDCs, as evidenced by

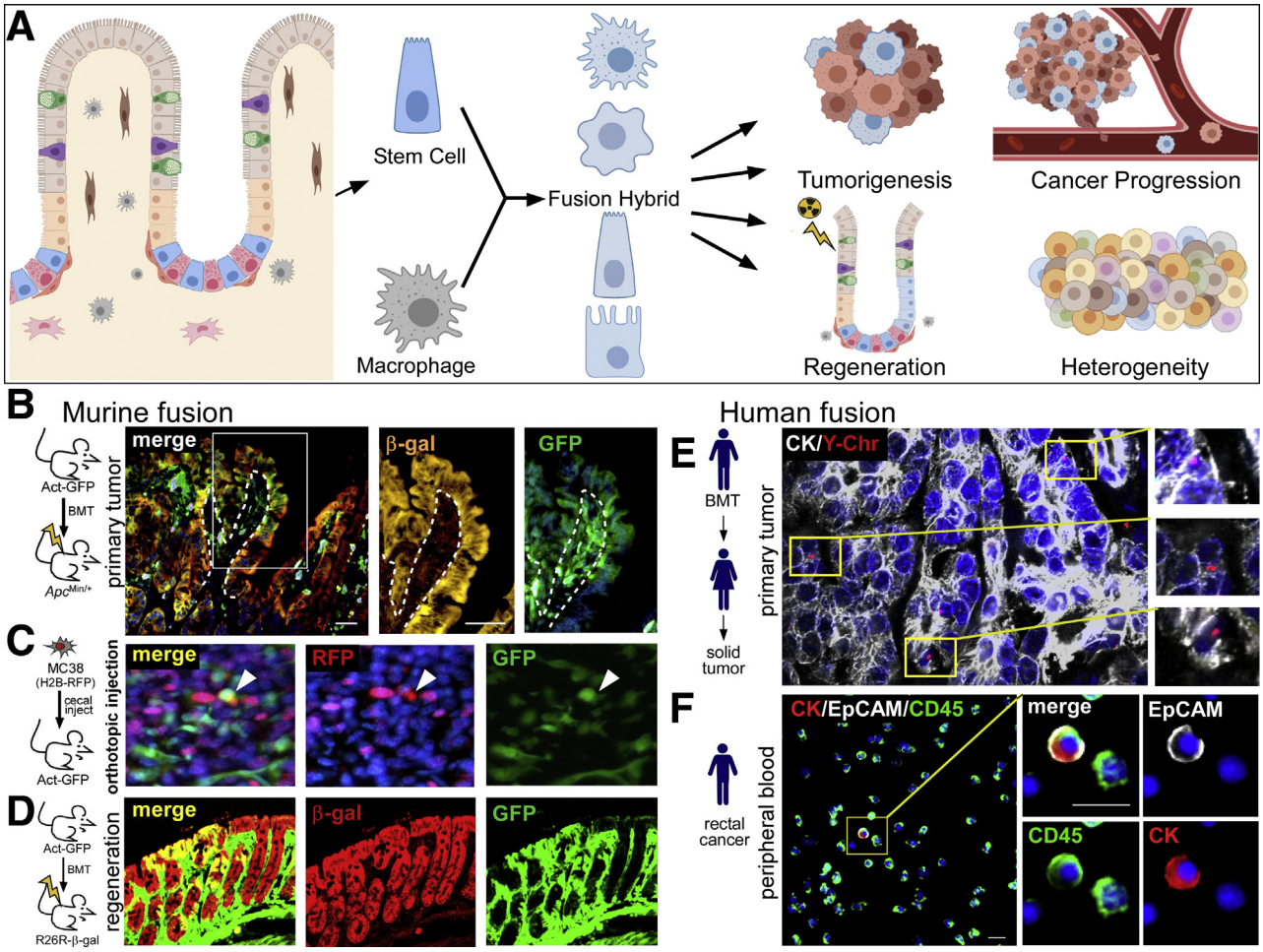

Figure 1. Regenerative and Pathologic Cell Fusion in Murine and Human Intestine. (*A*) Illustration overviewing the impact of functional cell fusion. (*B-D*) Cell fusion detected by co-expression of donor and recipient proteins using immunofluorescence microscopy in murine intestine. (*B*) Co-expression of β-gal and green fluorescent protein (GFP) within intestinal tumors of *Apc*$^{min/+}$/R26R-β-gal mice. Dashed line marks epithelial stromal boundary and white box is magnified to the right. (*C*) Red fluorescent protein (RFP)-marked colorectal cancer cells (MC38) injected into Actin-GFP mice grew tumors with detectible GFP^{+}/RPF^{+} co-expressing hybrids (arrowhead). (*D*) Fusion hybrids are also detected in injury repair following irradiation and bone marrow transplant (BMT) by the co-expression of β-gal and GFP. (*E*) In humans, cell fusion is detectible in pancreatic cancer tissue sections from a female recipient of sex-mismatched BMT by the co-expression of cytokeratin (CK, white) and a Y chromosome (Y-Chr, red), by fluorescence in situ hybridization. (*F*) From the peripheral blood of a human patient with rectal adenocarcinoma, circulating hybrid cells are detected by co-expression of CK (red), EpCAM (white) and CD45 (green). Bar = 25μm.

fusion phenotypes in all principal epithelial lineages within the same intestinal crypt or villus (Figure 1*C*), and the longevity of fusion hybrids through 14 months post-transplant, beyond the epithelial cell lifespan.[5] Cell fusion therefore may contribute to intestinal regeneration through the creation of functional, long-lived fusion hybrids.

Evidence of regenerative intestinal cell fusion in human beings is shown in female recipients of sex-mismatched BMTs.[6] After intestinal injury, numerous studies have reported detectable donor Y-chromosomes in up to 4.6% of examined intestinal epithelial cells.[7,8] Although the abundance of intestinal fusion hybrids does not prove their functional relevance, lessons from tumorigenesis indicate extensive genotypic and phenotypic heterogeneity among fusion-derived cells, which in itself is relevant. Heterogeneous epithelia could be protective against diverse threats to barrier integrity or impart differential engagement with immune cells. Barring this hypothesis, there is ample evidence of regenerative cellular fusion outside the intestinal tract, highlighted specifically in human hepatocytes and epidermis, as well as in murine hepatocytes, cardiomyocytes, retinal neurons, and skeletal myocytes after graft-versus-host disease, injury, or whole-body irradiation.[8–10] Numerous studies have corroborated fusion-mediated regeneration in multiple organ systems using a diverse set of methodologies, such as rescue from hepatotoxic fumarylacetoacetate hydrolase mutations[11] and restoration of both dystrophin expression and muscular function in *mdx* mice through BMDC or fibroblast transplantation, respectively.[12] A review of the large body of evidence on regenerative fusion is outside the

scope of this commentary, however it will suffice to say that there is substantial interest in harnessing fusion as a therapeutic modality.[13]

Maintenance of the intestinal epithelial barrier preserves survival of the organism; thus, redundancy in mechanisms underlying intestinal epithelial regeneration is beneficial and necessary. Homeostatic stem cell–mediated epithelial renewal is augmented by plasticity of differentiated cells, and by cell-cycle re-entry of quiescent or developmental stem cells to support epithelial proliferation.[14] Interestingly, there is evidence suggesting fusion may be an independent mechanism for inducing stem cell plasticity outside the intestine, perhaps owing to fusion-induced genomic changes.[13,15–17] It therefore is unsurprising that cell fusion represents an alternative regenerative mechanism in the intestine. Redundancy does not equate to unimportance, and regenerative fusion in the intestine should not be ignored because it is a ubiquitous and underappreciated mechanism with significance in a plurality of organ systems.

Future Directions for Cellular Fusion Research

Cellular fusion's impact on tissue regeneration and malignancy is wide-reaching, introducing functional diversity into homeostatic and diseased intestine. Although an independent functional role of fusion-mediated intestinal heterogeneity remains elusive, robust evidence in extraintestinal sites suggests that fusion represents a secondary regenerative pathway and has garnered interest for its potential therapeutic applications. In addition, there is compelling evidence in human beings and mice of the critical role that cellular fusion plays in gastrointestinal malignancies and the metastatic cascade by imparting heterogeneity and prometastatic characteristics to tumor cells. This interplay alone makes fusion a topic of immense interest in oncology. We look forward to a greater understanding of cellular fusion's impact on homeostasis and pathology, as well as the possibility of harnessing fusion in oncologic and regenerative medicine.

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