Role of tissue microenvironment resident adipocytes in colon cancer

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Abstract

Colorectal cancer (CRC) is a multifactorial disease characterized by several genetic and epigenetic alterations occurring in epithelial cells. It is increasingly recognized that tumour progression is also regulated by tumour microenvironment (TME). The bidirectional cross-talk between tumour resident adipocytes and cancer cells within TME has been proposed as active contributor to carcinogenesis. Tumour resident adipocytes exhibit an activated phenotype characterized by increased secretion of pro-tumorigenic factors (angiogenic/inflammatory/immune) which contribute to cancer cell proliferation, invasion, neoangiogenesis, evasion of immune surveillance and therapy resistance. Furthermore, adipocytes represent a fuel rich source for increasing energy demand of rapidly proliferating tumour cells. Interestingly, a relationship between obesity and molecular variants in CRC has recently been identified. Whether adipose tissue promotes cancer progression in subsets of molecular phenotypes or whether local tissue adipocytes are involved in inactivation of tumour suppressor genes and/or activation of oncogenes still needs to be explored. This editorial highlights the major findings related to cross-talk between adipocytes and colon cancer cells and how local paracrine interactions may promote cancer progression. Furthermore, we provide future strategies in studying colonic TME which could provide insights in...
bidirectional cross-talk mechanisms between adipocytes and colonic epithelial cells. This could enable to decipher critical signalling pathways of both early colonic carcinogenesis and cancer progression.

Key words: Tumour resident adipocytes; Dysfunctional adipocytes; Adipose tissue; Cancer cell-tumour resident adipocyte cross-talk; Colon cancer microenvironment

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Core tip: The tumor microenvironment (TME) has been implicated in cancer progression and chemoresistance. Adipocytes are active components of the TME. Bidirectional cross-talk between adipocytes and cancer cells has recently been postulated to actively contribute to tumor initiation and progression. This Editorial highlights the role of local paracrine interactions between adipocytes and colon cancer cells. Discovery of signalling pathways activated by tumor resident adipocytes in colon cancer will allow better understanding of carcinogenesis and provide potential therapeutic targets.

Tabuso M, Homer-Vanniasinkam S, Adya R, Arasaradnam RP. Role of tissue microenvironment resident adipocytes in colon cancer. World J Gastroenterol 2017; 23(32): 5829-5835 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i32/5829.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i32.5829

INTRODUCTION

Colorectal carcinogenesis is multifactorial involving interactions between genetic mutations (APC, TP53, PI3K, KRAS, BRAF, PTEN), microsatellite instability, chromosomal instability, epigenetic alterations (locus-specific CpG island methylation, global DNA hypomethylation) and environmental factors (obesity, diabetes, metabolic syndrome, intestinal microbiome). Moreover the importance of “field cancerisation” has been highlighted in terms of cancer development in the macroscopically normal colon. Epidemiologic studies support an association between obesity and molecular variants in CRC. It has been proposed that microenvironment-derived signals trigger heritable genetic changes within cancer cells, contributing to tumour evolution. Studies in breast cancer suggest that bidirectional interactions induce sequential epigenetic modifications in both cancer and stromal cells with progression from in situ ductal carcinoma to invasive carcinoma. Epigenetic modifications induced by tumour resident adipocytes in colon cancer cells have not been reported, although MPE studies have identified a relationship between obesity and molecular variants in CRC.

DYSFUNCTIONAL ADIPOCYTES AND CANCER

The main component of adipose tissue is white adipose tissue (WAT). Expansion of WAT is consequence of...
an increase in size (hypertrophy) and/or increase in number (hyperplasia) of adipocytes. Healthy adipose tissue expansion consists in hypertrophic and hyperplastic white adipocytes, with appropriate angiogenic response, extracellular matrix remodelling and minimal inflammation. In contrast, pathological expansion of adipose tissue consists of adipocytes hypertrophy resulting in hypoxia, reduced angiogenesis, infiltration of macrophages and immune cells, low-grade inflammation, excessive production of reactive oxygen radicals, endoplasmic reticulum stress, mitochondrial dysfunction and remodelling of extracellular matrix.

Inflammation is a recognised hallmark of cancer and pre-existing pro-inflammatory microenvironments are associated with increased cancer risk. Increasing evidence, in breast, prostatic, ovarian and colon cancer, suggests that dysfunctional adipocytes are involved in cancer cell proliferation and migration through dysregulation of local and systemic inflammatory-immune-angiogenic response system. Inflammation is initiated by adipose tissue hypertrophy leading to localized hypoxia which activates hypoxia-inducible factor 1-alpha (HIF-1α). HIF-1α up-regulates secretion of chemokines and proangiogenic factors including TNF-α, IL-6, IL-1, monocyte chemoattractant protein (MCP-1), plasminogen activator inhibitor-1 and VEGF, which are involved in the recruitment of macrophages and initiation of angiogenesis. Recruited macrophages contribute further to up regulation of inflammatory/immune cytokines favouring the acquisition of a systemic and local inflammatory phenotype.

**ROLE OF ADIPOSE TISSUE SECRETED FACTORS AND LIPID METABOLITES IN COLON CANCER**

The adipose tissue secreted factors, lipid metabolites and signalling pathways have been summarized in Table 1.

| AT secreted factors | Function | Signalling pathway | Ref. |
|---------------------|----------|--------------------|------|
| TNF-α               | Pro-inflammatory, cell proliferation, anti-apoptotic, angiogenic | PI3K, NF-κB | Pikarsky et al. [25], 2004 |
|                      |          |                    | Huang et al. [26], 2009 |
|                      |          |                    | Viatour et al. [27], 2005 |
|                      |          |                    | Hefetz-Sela et al. [28], 2013 |
|                      |          |                    | Hoda et al. [22], 2007 |
| IL-6                | Pro-inflammatory, cell proliferation and anti-apoptotic | JAK/STAT3 | Hodge DR et al. [24], 2009 |
|                      |          |                    | Hefetz-Sela et al. [28], 2013 |
| Leptin              | Promotion of cell survival, proliferation, differentiation, pro-inflammatory | JAK/STAT, PI3K, MAPK | Hoda et al. [22], 2007 |
| Adiponectin         | Anti-inflammatory, anti-proliferative and pro-apoptotic effect | Inhibition of PI3K, AMPK/mTOR, NF-κB | Hoda et al. [21], 2013 |
| Visfatin            | Anti-inflammatory, angiogenic, promotion of cell survival and migration, angiogenesis | ERK/MAPK, PI3K/AKT, NF-κB, β1-integrin | Hoda et al. [22], 2007 |
| Lipid peroxidation products | Promotion of cell proliferation, differentiation, survival, migration, angiogenesis | PI3K/AKT/mTOR, NF-κB, PPAR, MAPK | Ayala et al. [20], 2014 |

PI3K: Phosphoinositide 3-kinase; NF-κB: Nuclear factor-kappa B; JAK/STAT3: Janus kinase/signal transducers and activators of transcription 3; MAPK: Mitogen-activated protein kinases; AMPK: AMP-activated protein kinase; mTOR: Mammalian target of rapamycin; HIF-1α: Hypoxia-inducible factor 1-alpha; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin-6; AKT: Protein Kinase B; ERK: Extracellular signal-regulated kinase; PPAR: Peroxisome proliferator-activated receptors.

**TNF-α**

TNF-α, secreted by dysfunctional adipose tissue, has been shown to support cancer cell proliferation, angiogenesis and metastasis through activation of key transcription factors, including PI3K/AKT/mTOR and nuclear transcription factor NF-κB. TNF-α and hypoxic conditions also induce secretion of the proinflammatory cytokine IL-6, activator of Janus Kinase and signal transducers and activators of transcription 3 (Jak/STAT3) pathways, key regulators of cell proliferation and apoptosis.

Adipoxy secreted hormones, including leptin, adiponectin and visfatin, have also been implicated in colon cancer progression.

**Leptin**

Leptin is a potent inflammatory agent involved in up regulation of pro-inflammatory cytokines such as TNF-α, MCP-1, and reactive oxygen species from endothelial cells and peripheral blood mononuclear cells. In vitro studies, in colon cancer cell lines, have demonstrated that leptin exerts pro-inflammatory, mitogenic, anti-apoptotic and angiogenic properties.

**Adiponectin**

Adiponectin has a potent anti-inflammatory, anti-proliferative and anti-apoptotic activity. However, proliferative and pro-inflammatory properties of adiponectin on colonic epithelial cancer cells have also been reported. Several studies suggest local-paracrine pro tumorigenic effects of adiponectin according to tissue-specific expression of its receptor subtypes (ADIPOR1 and ADIPOR2). Increased AdipoR1 and AdipoR2 expression has been associated with cancer progression linked with the pro-angiogenic activity of adiponectin.

**Visfatin**

Visfatin has been shown to exhibit pro-inflammatory
and pro-angiogenic effects in endothelial cells\cite{25}. Studies have demonstrated a role of visfatin in CRC. CRC cells express chemokine receptors (CXCR4 and CXCR7), activated by visfatin, which bind stromal cell-derived factor-1, promoter of survival and migration of cancerous cells\cite{26}.

**Lipid peroxidation products**
The chronic low-grade inflammatory state of dysfunctional adipocytes leads to activation of lipid peroxidation with the production and secretion of 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde. The secreted 4-HNE is responsible of deregulation of multiple pathways involved in tumour cell proliferation, differentiation, cell survival, apoptosis and angiogenesis including MAPK, PI3K-AKT-mTOR, NF-kB. This also results in upregulation of prostaglandin E2 (PGE2) and cyclooxygenase-2, implicated in CRC\cite{27}.

### ACTIVATED PHENOTYPE OF TUMOUR RESIDENT ADIPOCYTES AND LOCAL PARACRINE REGULATION OF COLON CANCER

Recently great interest has emerged in reciprocal signalling between tumour resident adipocytes and cancer cells. CRC progresses through sequential stages involving multiple layers of the colonic wall. TNM staging system is currently used for classifying CRC in 4 stages according to local invasion depth (T stage), lymph node involvement (N stage) and presence or absence of distant metastasis (M stage), providing indication for prognosis and therapeutic strategies. With cancer progression activation of complex signalling networks modify both cancer cells and stromal cells\cite{28}. Cancer cells and activated stromal cells communicate by autocrine/paracrine pathways contributing to dynamic modulation of TME through persistent recruitment of inflammatory and stromal cells in the TME. As a result, TME becomes increasingly populated with infiltrating innate immune cells (macrophages, neutrophils), adaptive immune cells (T and B lymphocytes) pericytes and stem cells contributing to cancer cell proliferation and invasion (Figure 2).

**Figure 1** Signalling pathways activated by tumour resident adipocytes secreted factors. Tumour resident adipocytes secreted factors activate cell cycle regulators and inflammatory/immune/angiogenic regulators. Cancer cell secreted inflammatory cytokines activate host cells of TME constituting a paracrine/autocrine loop. MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3-kinase; AKT: Protein Kinase B; mTOR: Mammalian target of rapamycin; NF-kB: Nuclear factor-kB; JAK/STAT3: Janus kinase/signal transducers and activators of transcription 3; HIF-1α: Hypoxia-inducible factor 1-alpha; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin-6.

| Colon cancer microenvironment | Tumor resident adipocyte |
|-------------------------------|-------------------------|
| Cytokines/growth factors | Adipokines | Products of lipid peroxidation |
| HIF-1α | PI3K/AKT | MAPK |
| JAK/STAT3 | AKT | Cell cycle regulators |
| NF-kB | Inflammatory/immune regulator |
| MAPK | NF-kB | Angiogenic regulator |
| JAK/STAT3 | HIF-1α |

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activity, decreased adipocyte-differentiation markers (adiponectin, resistin, fatty acid binding protein-4, adipocyte protein 2), and increased secretion of inflammatory factors (IL-6, IL-8, IL-1β, TNF-α), growth factors (insulin-like growth factor 1, IGF1 binding proteins), angiogenic factors (VEGF) and MCP-1 (CCL2) [15]. In vitro studies, in breast and prostate cancer, have demonstrated that tumour resident adipocyte secreted factors activate signalling pathways involved in cancer cell survival, proliferation, invasion, epithelial to mesenchimal transition, angiogenesis and extracellular matrix remodelling, promoting cancer initiation and metastasis [15,22]. Active recruitment of adipocytes to TME has not been reported, although it has been reported that bone marrow derived mesenchymal stem cells (progenitors of adipocytes) may be recruited to specific sites of neoplasia inducing metastatic properties [29]. An open question is whether adipose tissue promotes cancer progression in subsets of molecular phenotypes or whether local tissue adipocytes are involved in inactivation of tumour suppressor genes and/or activation of oncogenes (Figure 3).

Adipocytes serve as a fuel rich source for increasing energy demand of rapidly proliferating tumour cells. Advanced stages of gastrointestinal malignancies often present with cancer-associated-cachexia as a result of lipolysis induced by cancer cells. Studies have described increased lipid droplets in colon adenocarcinoma and it has been implicated in PGE2 synthesis. Inhibition of lipid droplet formation by fatty acid synthase inhibitors reduces cancer cell proliferation in vitro, suggesting a role of lipid droplets in colon adenocarcinoma [30].

Metabolic and transcriptomic expression profile and direct paracrine effects of tumour resident adipocytes in colon cancer have not been evaluated. We have preliminary data (unpublished) indicating increased expression of pro-inflammatory/immune/angiogenic factors in colon cancer resident adipocytes, isolated from paraffin embedded sections using laser micro dissection system, compared to adipocytes isolated from the distal non neoplastic mucosa.

TME signalling pathways have recently been implicated in inducing chemoresistance in breast and prostate cancer [31]. There is also evidence, in colon cancer cell lines, that leptin inhibits cytotoxic effects of 5-fluorouracil [32].

**Novel strategies in studying TME**

Two dimensional (2D) cell culture models, widely used in basic science research, do not reproduce the complex interactions between host cells of TME. Recently, three-dimensional (3D) organoid models, derived from mouse and human intestinal tissue ex vivo, have been described. These in vitro organ-like cultures reproduce intestinal tissue microenvironment. Furthermore, they can be co-cultured with stromal components [33]. Reproduction of colonic microenvironment in vitro will allow to decipher ex vivo bidirectional cross-talk mechanisms between adipocytes and colonic epithelial cells.
CONCLUSION

The bidirectional cross talk between tumour resident adipocytes and colon cancer cells contributes to the progressive evolution of tumour microenvironment and cancer progression. It is therefore important to decipher the metabolic and transcriptomic expression profiles of colon cancer resident adipocytes in different stages of tumour progression. Colon organoid cultures combined with adipocytes and/or tumour resident adipocyte secreted factors will allow to identify critical signalling pathways of both early colonic carcinogenesis and cancer progression providing diagnostic biomarkers and novel therapeutic targets for colon cancer.

ACKNOWLEDGMENTS

We would like to thank Jason McAllister for the creation of the figures.

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P- Reviewer: Abdel-Rahman WM, Harmanci O, Zhu YL
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