



## Mesenchymal stem cells in the colorectal tumor microenvironment: Recent progress and implications

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**Title:**

Mesenchymal Stem Cells in the Colorectal Tumour Microenvironment – recent progress and implications.

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**Abbreviations:** MSC: mesenchymal stem cell; CAF: carcinoma associated fibroblast; FAP: fibroblast activation protein; EMT: epithelial-mesenchymal transition

**Keywords:** mesenchymal stem cells; carcinoma associated fibroblasts; epithelial mesenchymal transition; tumour microenvironment; colorectal cancer

**Novelty and impact of the paper:**

MSCs are known to specifically migrate to and engraft within colorectal tumours, integrating into the architecture of the primary tumour microenvironment.

Considering the established importance of inflammation in colorectal cancer and the known role of stromal cells in this process, there is a potential wealth of information to be gleaned from understanding interactions between MSCs and colorectal cancer cells in this context. Epithelial-mesenchymal transition is central to colorectal cancer progression and MSCs have also been implicated in this process. This is the first review of MSC-colorectal cancer cell interactions, highlighting the strengths and weaknesses of recently published data, and placing this knowledge in the context of the current state of the art in this rapidly expanding field.

**Abstract:**

Mesenchymal stem cells (MSCs) are non haematopoietic multipotent adult stem cells. They have been shown to have a natural tropism for many tumours types, including colorectal, and are capable of escaping host immune surveillance. MSCs are known to engraft at tumours and integrate into their architecture, potentially as carcinoma associated fibroblasts (CAFs). In contrast with other malignancies, our understanding of the interactions between colorectal cancer cells and MSCs remains limited. Considering the established importance of inflammation in the colorectal cancer primary tumour microenvironment and the role of stromal cells in this process, there is a potential wealth of information to be gleaned from further investigation of interactions between these cell populations. Epithelial-mesenchymal transition is central to colorectal cancer progression and MSCs have also been implicated in this process. This review explores the current knowledge (both *in vitro* and *in vivo*) of interactions between colorectal cancer cells and MSCs. It highlights potential effects of cell source, number and ratio on outcome of *in vivo* studies, and explores strategies to more accurately explore their role in the primary tumour microenvironment. As our understanding of the underlying molecular processes in colorectal cancer develops, elucidation of these interactions will be central to development of novel therapeutic strategies for this prevalent disease.

## **Introduction**

It is estimated that in 2008, colorectal cancer was responsible for 8% of all cancer deaths, making it the fourth most common cause of death from cancer<sup>1</sup>. The major cause of death in this malignancy is development of metastasis in liver, abdominal lymph nodes, and lung, for which there is no cure<sup>2</sup>. Prognosis is heavily related to stage at diagnosis. According to the most recently defined American Joint Committee on Cancer (AJCC) system for colonic adenocarcinoma, 5-year stage-specific survivals were 93.2% for stage I disease compared with 8.1% for stage IV<sup>2</sup>. This highlights a need to expand our relatively limited knowledge of primary colorectal tumour biology with the ultimate goal of developing novel therapeutic strategies.

Tumours consist of an environment rich in inflammatory cells, immune cells, tumour vasculature, extracellular matrix and stromal cells. While carcinomas are derived from epithelial cells, the role of the stroma in this complex microenvironment has been afforded increasing prominence in recent years<sup>3</sup>. The genotype and phenotype of the cancer cell is intrinsically linked to the environment in which it resides and it provides the lifeblood which allows the tumour to flourish. The proliferation of the tumour is therefore dependent on stromal elements. The potential clinical implications of the role of stromal elements in the colorectal tumour microenvironment has been emphasised by Mesker et al<sup>4</sup> who reported the epithelial–stromal ratio of colon carcinomas to be a predictor of survival independent to lymph node status and tumour stage. As a result, this area represents an exciting potential target for therapeutic intervention<sup>5</sup>.

## **Mesenchymal Stem Cells (MSCs)**

### ***Biology and characterisation***

Mesenchymal Stem Cells (MSCs) are a remarkable subset of stromal cells. They are non-hematopoietic, multipotent cells which make up a very small percentage (0.01%) of the population of nucleated cells in the bone marrow<sup>6</sup>. They are known to be actively recruited to the site of wounds or areas of chronic inflammation. Both cultured rat and human MSCs have been transplanted intravenously in rat models and shown to migrate into sites of brain injury after cerebral ischemia<sup>7</sup>. Other groups have documented uptake of MSCs in infarcted myocardium after infusion of cultured MSCs in rats<sup>8</sup>. MSCs were observed to be preferentially attracted to, and retained in, ischemic tissue but not in the remote or intact myocardium. Due to their ability to home to sites of injury, MSCs have also generated a great deal of interest because of their potential use in regenerative medicine, particularly in spinal cord injury<sup>9</sup>. MSCs are known to take part in colonic mucosal regeneration<sup>10</sup> and recently, transplanted MSCs have been associated with accelerated healing of experimentally induced colitis in animal models<sup>11, 12</sup>.

Uniform characterisation of MSCs was deemed necessary due to the rapidly increasing biological and clinical interest in these cells over the past years. Therefore, MSCs, by definition<sup>13</sup>, are plastic adherent cells which express a characteristic panel of antigens including CD105, CD73 and CD 90 in >95% of culture. They are also devoid of CD14, CD34, CD 19, HLA DR and CD45. MSCs must also possess the ability to differentiate into osteoblasts, adipocytes and chondroblasts<sup>13</sup>. This capacity for trilineage mesenchymal differentiation is arguably the biologic property that most uniquely identifies MSCs. They are readily extractable from bone marrow but may

also be isolated from other mature tissues such as skeletal muscle, umbilical cord, amniotic fluid and adipose tissue<sup>14-16</sup>.

### **MSCs in Colorectal Cancer**

It has been established that MSCs migrate to the tumour stroma of colorectal cancer and other cancers<sup>17, 18</sup>. The migratory pathway is dependent on a multitude of signals ranging from growth factors to chemokines secreted by injured cells or immune cells<sup>19</sup>. The mechanism of recruitment of MSCs from bone marrow to tumours has been shown to exhibit significant overlap with the migration and activation of inflammatory cells in tissue repair processes<sup>20</sup>. While little evidence exists specifically in relation to the role of factors in MSC migration to colorectal tumours, a dose dependant response of MSCs to a variety of factors has been shown in other malignancies. These include monocyte chemotactic protein-1 (MCP-1/CCL2)<sup>21</sup>, growth factors with strong immunogenic properties including epidermal growth factor (EGF)<sup>19</sup>, RANTES (CCL5)<sup>22</sup>, CXCL8<sup>23</sup>, and receptors such as CD44<sup>24</sup>. Further elucidation is required specifically in relation to colorectal cancer. Chamberlain et al<sup>25</sup> concluded it may be reasonable to accept that MSC migration to the tumour is regulated through integrins and adhesion molecules, similarly to the well described process of leukocyte migration. Principles relating cancer to chronic inflammation were initially outlined by Haddow<sup>26</sup> in 1972 and emerging similarities led Dvorak<sup>27</sup> to describe tumours as “wounds that never heal”. Stromal constituents are central characters in the process of inflammation, providing us with key evidence in establishing the mechanism behind the long-recognized link between malignancy and inflammation. Inflammatory cells are a prominent component of the stroma of

carcinomas whether or not they have arisen secondary to a chronic inflammatory condition such as Ulcerative Colitis<sup>28</sup>.

### ***Potential precursors for Carcinoma Associated Fibroblasts***

Initial elucidation of the remarkable properties of MSCs led to burgeoning interest in their use as potential vectors for delivering therapeutic targets to the site of tumours<sup>29</sup>.

However, it has become abundantly clear that we first need to expand our limited knowledge of the interaction between MSCs and cancer cells. It has been established that MSCs migrate to the tumour stroma of colorectal cancer<sup>17, 18</sup>. In the context of many cancers, concerns have been raised regarding a potentially deleterious effect of MSCs through protumorigenic activity or consequences of immune suppression.

Studies have addressed this issue in relation to breast<sup>30, 31</sup>, colorectal<sup>17</sup> and other cancers with conflicting conclusions. In this review, the focus will remain on colorectal cancer.

It has been hypothesized that MSCs are potential precursors for Carcinoma Associated Fibroblasts (CAFs). CAFs play a key role in tumour progression providing a supportive environment for growth, and CAFs expressing fibroblast activation protein (FAP) have been implicated in the invasive behaviour of colorectal cancer<sup>32, 33</sup>. Shinagawa et al observed that, following systemic injection via the tail vein, the morphology and distribution of MSCs within colorectal tumour stroma were similar to those of CAFs<sup>17</sup>. MSCs which were functionally incorporated into the colorectal cancer tumour stroma expressed alpha smooth muscle actin ( $\alpha$ -SMA), beta-type platelet-derived growth factor receptor (PDGFR- $\beta$ ), desmin, FAP and Fibroblast Surface Protein (FSP). Furthermore, Henriksson et al<sup>33</sup> published evidence of communication between cancer cells and fibroblasts leading to colorectal cancer cell

invasion using FAP expression as a marker of fibroblast activation. They analyzed the effect of activated fibroblasts on colorectal cancer migration and invasion in experimental cell studies. The expression pattern of FAP in CAFs during transformation from benign to malignant colorectal tumours was scrutinized. Interestingly, they found through immunohistochemical analyses that FAP was expressed in fibroblasts of all colorectal cancer samples examined, whereas all normal colon, hyperplastic polyps, or adenoma samples were negative for the protein. Activated fibroblasts were shown to increase the migration and invasion of colon cancer cells in three dimensional culture. Fibroblast Growth Factor 1/Fibroblast Growth Factor Receptor 3 (FGF1/FGFR-3) signalling was shown to lead to increased migration and invasion. The myofibroblast cell line utilised in this study, CCD-18Co, was obtained from the American Type Culture Collection and is known to be plastic adherent although the panel of cell surface antigens expressed was not available. A proposed method of reducing migration of colon cancer cells through addition of a fibroblast growth factor receptor inhibitor and an FGF1-neutralizing antibody requires further investigation <sup>33</sup>. In summary, Shinagawa et al report that MSCs within the colorectal cancer stroma are similar to CAFs and Henriksson et al elucidate expression patterns of FAP in CAFs during transformation from benign to malignant colorectal tumours. Taken in combination, these studies provide mounting evidence regarding the potential role of MSCs in the colorectal tumour microenvironment. Nakagawa et al <sup>32</sup> have attempted to further investigate the molecular features of cancer stroma using fibroblast cell cultures from metastatic colon cancer. Samples were isolated from liver tumour, tumour associated normal liver and skin from three patients with metastatic colorectal cancer. They then used oligochip arrays to generate expression profiles of cancer-associated fibroblasts and compare them to those of

fibroblasts uninvolved in a malignant process. They found that conditioned media from these cancer-associated fibroblast cultures enhanced proliferation of colon cancer cell line HCT116 to a greater degree than cultures from uninvolved fibroblasts. Using immunohistochemistry ex-vivo, they also confirmed COX2 and TGF $\beta$ 2 expression in cancer-associated fibroblasts in metastatic colon cancer<sup>32</sup>. Fibroblast cultures were established from three sources (metastatic liver lesion, associated normal liver away and skin) in three patients with metastatic colorectal cancer in the liver. Absence of epithelial cells was confirmed by reverse transcriptase–polymerase chain reaction (RT–PCR) of epithelium specific markers (cytokeratin-19 and -20). The group confirmed that the cultures were composed of fibroblasts by RT–PCR for vimentin and immunofluorescent staining for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). It must be noted, however, that Shinagawa et al reported that MSCs which were functionally incorporated into the colorectal cancer tumour stroma also expressed alpha smooth muscle actin ( $\alpha$ -SMA)<sup>17</sup>. Furthermore vimentin is a marker for mesenchymal lineage cells which MSCs are positive for<sup>34</sup>. Therefore, it is plausible that at least a percentage of cells reported to be CAFs by this group, could in fact be MSCs. Regardless, this elucidation of distinct molecular expression profiles of CAFs in colon cancer metastasis supports the hypothesis that fibroblasts form a supportive microenvironment for cancer cells and highlight the importance of the discovery that MSCs represent potential precursors for CAFs.

### ***Impact on Colorectal Cancer Development and Progression***

While the effect of colonic myofibroblasts and other fibroblasts on enhancing tumour formation has been investigated previously<sup>35,36</sup>, until recently no evidence was available regarding the effect of MSCs on colorectal tumour sphere formation and

initiation. Admixed MSCs have now been reported to enhance the ability of colorectal tumour cells to form spheres *in vitro*<sup>37</sup>. In the same study, MSC-derived conditioned medium was shown to enhance tumour sphere formation suggesting the involvement of paracrine factors<sup>37</sup>. Moreover, reports from a recent study using a novel 3D co-culturing system, showed that fibroblasts positive for the mesenchymal marker vimentin induced an invasive phenotype in spheroids of LS174T colorectal cancer cells<sup>38</sup>, further supporting a role for MSCs in tumorigenesis.

While recent literature sheds some light on the migration, invasion and proliferation of colorectal cancer cells, the effect, if any, of MSCs on this process remains poorly understood. Recent research has focused on the effect of MSCs on growth and metastasis of the disease *in vivo*. A recent study suggests that human bone marrow derived MSCs enhance the growth and metastasis of colon cancer<sup>17</sup>. The MSCs employed were confirmed to be plastic adherent, with multilineage differentiation capacity and expression of appropriate cell surface antigens confirmed to fulfil minimum criteria as previously described. Firstly, this group set out to confirm that MSCs migrate to the site of colorectal tumours using a murine model of human colon cancer. Eight-week-old female athymic mice underwent transplantation of  $1.0 \times 10^6$  KM12SM human colon cancer cells into their caecal wall. On day 21, they received a systemic injection (via the tail vein) of  $1.0 \times 10^6$  PKH26-labeled MSCs. Seven days later, the mice were sacrificed and fluorescently labelled MSCs were detected in the tumour stroma by fluorescence microscopy, but were not observed in normal surrounding tissue. The study then proceeded to examine the effect of MSCs on tumour growth at the orthotopic site using co-injection studies. To this end, mice were divided into three groups. The first group underwent intracaecal injection of

KM12SM cells alone ( $0.5 \times 10^6$  cells). The second group were injected with KM12SM cells mixed with MSCs (KM12SM:MSCs –  $0.5 \times 10^6$ : $1.0 \times 10^6$  cells- ratio 1:2). The final group received MSCs alone ( $1.0 \times 10^6$  cells). Tumour weight, incidence of liver metastasis and survival rates were evaluated. In the group which received orthotopic transplantation of KM12SM cells mixed with MSCs greater tumour weight was reported than in the group which received transplantation of KM12SM cells alone. Survival rate was significantly reduced in the animal group who received mixed cells. Enhanced growth and macroscopic liver metastases were seen only in this group. The ratio of MSCs:CRC cells used in *in vivo* studies is likely to be a critical determinant in solving current controversies as to whether MSCs have a pro- or anti-tumorigenic role in the colorectal cancer stroma. Although the *in vivo* ratio of MSCs to tumour cells in the tumour microenvironment has not yet been established, it is known that MSCs make up only 0.01% of the population of nucleated cells in the bone marrow<sup>6</sup>. Armed with this knowledge, is it reasonable to hypothesize that a ratio of MSCs:CRC of 2:1 is likely to be physiologically relevant?

It is also crucial to establish whether enhanced growth would also be observed if MSCs were replaced with the same number of normal fibroblasts. Would any cell secreting these factors cause these effects or is it an MSC related phenomenon?

Another co-injection study by Tsai and colleagues addressed both variations in ratio and inclusion of normal fibroblast controls with interesting results<sup>37</sup>. They performed co-injection studies of human colorectal cancer cells, MSCs, and a mixture of both cell types in immunodeficient mice and concluded that MSCs promote formation of colorectal tumours<sup>37</sup>. HT 29 human colorectal cancer cells were mixed with human bone marrow derived MSCs at varying ratios, injected subcutaneously without matrigel and their ability to form tumours in mice quantified. MSCs were

characterised to meet minimum definition criteria including plastic adherence, expression of MSC surface antigens and capacity to differentiate into osteoblasts, adipocytes and chondrocytes. Although injection of colorectal cancer cells (HT-29) alone induced tumour formation with  $1 \times 10^5$  cells (83% within 3 weeks) or  $1 \times 10^6$  cells (100% within 1 week), no tumours were formed with only  $1 \times 10^4$  cells even after a 6 month time period. Interestingly, however, when the same number ( $1 \times 10^4$ ) of HT-29 cells were mixed with  $9 \times 10^4$  MSCs (ratio 9:1 MSC:CRC), tumour formation occurred within 2 to 3 weeks. However, this effect was also noted with WI38 normal lung fibroblasts and primary fibroblasts. Although this suggests that this is not an MSC-specific phenomenon, the ability of MSCs to enhance tumour initiation or sphere formation was significantly higher than normal or tumour colonic myofibroblasts<sup>37</sup>. Importantly, MSCs alone could not form tumours with cell numbers of up to  $2 \times 10^6$  nor could lower ratios of MSC to CRC (1:1, 1:3, 1:9)<sup>37</sup>. The results of these in vivo studies must be interpreted in the context of total cell numbers and ratio of MSCs to cancer cells. In the Shinagawa study, it must be noted that the group which received a mixture of MSCs and colon cancer cells were injected with three times the total number of cells than the group which received colon cancer cells alone. Therefore the question must be raised as to whether at least part of the increased tumour bulk was attributed simply to the fact that three times the number of cells was administered? Tsai et al reported increased tumour initiation at the highest ratio used in their study (9:1 MSCs to colorectal cancer cells). It does not appear reasonable to report that MSCs promote growth of colon cancer based on these ratios of MSCs to colon cancer cells. Perhaps more physiologically relevant outcomes could be reached by using ratios more likely to be representative of the in vivo environment.

It is noteworthy that at lower ratios of MSCs to colorectal cancer cells no pro-tumorigenic role was observed<sup>37</sup>.

However, a recent study by Liu and colleagues selected a much lower ratio of 10:1 CRC:MSC and reported that MSCs may be capable of directly contributing to tumorigenesis in colorectal cancer through production of key inflammatory factors after becoming incorporated into the tumour stroma<sup>18</sup>. C26 murine colon cancer cells and murine bone marrow derived MSCs were prepared either as single-cell type suspensions ( $1 \times 10^6$  cells) or in combination ( $1 \times 10^6$  C26 cells and  $1 \times 10^5$  MSCs ratio 10:1). MSCs were confirmed to be plastic adherent, capable of differentiating into adipocytes and osteoblast-like cells and confirmed by flow cytometry to be positive for CD90, CD105, CD166, CD44, and CD29 and negative for CD34, CD14, and CD45.

Some groups of MSCs were pre-treated with IFN- $\gamma$  or TNF- $\alpha$ . The C26 cell groups were administered subcutaneously (alone or mixed with MSCs) into the armpit areas of mice. Mice were examined three times per week and euthanized on day 18. Tumour growth was evaluated by measuring the length and width of the tumour mass. They reported that relative to C26 alone controls, C26 cells co-injected with MSCs were found to result in more tumour growth *in vivo*. They demonstrated that when MSCs were pre-stimulated by inflammatory factors (IFN- $\gamma$  and TNF- $\alpha$ ) prior to systemic injection, they could promote tumour angiogenesis *in vivo* to a greater degree than untreated MSCs or MSCs pre-stimulated by either inflammatory factor in isolation. Tumour angiogenesis has long been recognised as a key step in tumour growth. This study highlighted the potential pro-angiogenic effects of MSCs in colorectal tumour tissues. This group reported evidence that MSCs produce and

secrete pro-angiogenic cytokines such as VEGF, thereby promoting angiogenesis and potentially contributing to colon cancer growth through this direct mechanism.

The growth factor signaling pathway involved in tumor initiation, and the potential role of MSCs in this environment is still poorly understood. IL-6 has been implicated in this setting with tumour cells shown to stimulate MSC secretion of the cytokine.<sup>37</sup>. This in turn stimulated tumour cell proliferation. Tumour growth was proposed to be mediated through microvascularization, stromal network involvement and the production of tumour-stimulating paracrine factors. Tsai and colleagues also reported that an antibody against IL-6 or lentiviral-mediated transduction of an interfering RNA against *IL-6* in MSCs disrupted this process by preventing the ability of MSCs to promote sphere formation and tumour initiation.

In furthering our understanding of the behaviour of MSCs and colorectal cancer cells, it is crucial to reach a consensus on the issue of ratio of MSCs to cancer cells. Only when we have reached equipoise on this issue, can we definitively say that any of the effects described are physiologically relevant. Inclusion of appropriate stromal/fibroblast controls is key to determining whether effects described are MSC specific. Outside of these studies, there is no evidence in the literature about the effect of variations in ratio of MSCs to colorectal cancer cells, however, broadening the scope to other malignancies yields enlightening reports. Maestroni et al conducted an in vivo study using MSCs mixed with B16 melanoma cells in a ratio of 2:1(MSCs : Tumour Cells) in a murine model and reported inhibition of primary tumour growth and metastasis<sup>39</sup>. Conversely, it was later reported by Djouad et al that subcutaneous injection of B16 melanoma cells with MSCs resulted in a promotion of tumour growth after co-injection of MSCs and tumour cells in the ratio of 1:1<sup>40</sup>. Most

recently, Suzuki et al used various ratios of MSCs with B16-LacZ cells/LLC cells, lung carcinoma cell lines, to assess the effects of MSCs on tumour promotion and growth in an *in vivo* model<sup>41</sup>. Mixtures of each of these cell types, along with MSCs, at ratios of 1:0.2, 1:1 and 1:5, (Tumour Cells:MSCs) were subcutaneously injected in a murine model, with interesting results. At day 21 after tumour inoculation, mice injected with B16-LacZ cells and MSCs at 1:1 and 1:5 ratios exhibited 2.3-fold ( $P < 0.01$ ) and 4.3-fold ( $P < 0.01$ ) greater tumour volumes, respectively, than mice injected with B16-LacZ cells alone. In contrast, however, the group who received an injection of cells at a ratio of 1:0.2 did not show increased tumour size compared with B16-LacZ cells alone. On day 13 after tumour inoculation, mice injected with LLCs and MSCs at 1:1 and 1:5 ratios exhibited 2.1-fold ( $P < 0.05$ ) and 2.6-fold ( $P < 0.01$ ) greater tumour volumes respectively, when compared with mice injected with LLCs alone. Injection of MSCs alone did not result in tumour formation. These studies highlight the importance of ratio and total cell number in accurately assessing effect of MSCs on tumour growth<sup>42</sup>. Further investigation on the behaviour of MSCs and colorectal cancer cells in different ratios is therefore crucial to elucidation of the intricacies of their complex relationship.

### ***Potential Role in Stimulating Epithelial Mesenchymal Transition (EMT)***

Colon cancer is a molecularly heterogeneous disease and consequently a malignancy for which it is difficult to isolate any one unifying molecular hypothesis of biology and behaviour. Unlike breast cancer, in which intrinsic subtypes such as basal and luminal have been described<sup>43</sup>, colon cancer has yet to be classified by unsupervised, molecular profiling approaches. In recent months, however, Loboda et al published a study describing unsupervised analysis of the most variable genes expressed in 326

human colon cancer samples with the aim of exploring the intrinsic biology of colon cancer<sup>44</sup>. They concluded that the most dominant pattern of intrinsic gene expression in colon cancer was tightly correlated with the EMT signature. This interesting data suggests that this malignancy may potentially be resolved into two principal molecular subtypes of colon cancer: epithelial or mesenchymal<sup>44</sup>.

Invasion by colorectal carcinomas is characterized by an epithelial-mesenchymal transition (EMT)<sup>45,46</sup>. Interestingly, MSCs have been reported to stimulate EMT in breast cancer cell lines in vitro<sup>47</sup>. EMT is a complex morphogenetic process in which epithelial cells lose their innate characteristics and gain new mesenchymal properties.

In the context of normal physiology, EMT affects tissues as a coordinated unit.

During embryogenesis, for example, EMT enables the development of mesoderm from epithelium in the gastrulation phase<sup>48</sup>. However, in the context of malignancy EMT facilitates acquisition of a migratory phenotype by malignant cells thereby promoting tumour invasiveness<sup>49</sup>. Oncogenic EMT occurs in conjunction with other abnormalities intrinsic to cancer cells and, as described, is an integral component of colorectal cancer progression<sup>45,46,50</sup>. It is thought to endow cancer cells with migratory, invasive, and stem cell properties by activating a variety of signalling pathways and their target genes. The main pathway in colorectal cancer is the Wnt pathway effector  $\beta$ -catenin, which is over expressed due to mutations in the Adenomatous Polyposis Coli (APC) tumour suppressor in most cases<sup>51</sup>. The EMT process can be thought of as “dedifferentiation”.

It is thought that EMT may be mediated by surrounding cells. No evidence is currently available in the literature to link MSCs to this process in colorectal cancer cells. However, in a breast cancer model, Martin et al reported that MSCs may facilitate metastasis by promoting EMT<sup>47</sup>. Interestingly, they found that following

direct co-culture with MSCs, breast cancer cells expressed elevated levels of oncogenes, proto-oncogenes, genes associated with invasion, angiogenesis and anti-apoptosis. Conversely, universal down regulation of genes associated with proliferation was observed, with resultant decreased ATP production in response to MSC-secreted factors. This profile was consistent with EMT. Further investigation revealed significant up regulation of EMT specific markers (N-cadherin, Vimentin, Twist and Snail) following co-culture with MSCs, with a reciprocal down regulation in E-cadherin protein expression. These changes appeared to be MSC specific with the majority being cell contact mediated. Considering the established link between EMT and colon cancer, and the fact that MSCs have been shown to integrate into the colorectal cancer stroma, the role of EMT in colorectal cancer merits further elucidation.

## **Conclusion**

MSCs home to colorectal tumours and integrate into their architecture, with very little known regarding their impact following engraftment. Recent studies suggest they may play a pro-tumorigenic role through differentiation into CAFs or secretion of key inflammatory factors. However, significant variations in effect have been observed with differing cell number, source and ratio. In the limited studies performed to date, increased colorectal tumour growth and enhanced metastatic potential has been observed in the presence of MSCs. These studies employed MSCs mixed with colorectal tumour cells for tumour establishment, with a vast excess of MSCs to tumour cells present. Although informative, this is unlikely to accurately represent the in vivo situation. Considering the established tumour-homing abilities of MSCs, these cells represent a potential novel therapeutic vector for a disease with a paucity of

options for patients in whom distant metastasis has occurred. The future of developing MSC based therapies, or indeed targeting MSCs themselves in colorectal cancer lies in a more thorough understanding of MSC/colorectal cancer cell interactions. While important progress has been made in the past twelve months, future *in vivo* studies must pay stringent attention to definition of MSC characteristics and delineation of cell source. Experimental design must strive for cell numbers and ratios which more closely recapitulate the primary tumour microenvironment to clarify the role of MSCs in this setting. Despite very rare reports of human MSC transformation following long-term passage *in vitro*<sup>52, 53</sup>, the majority of studies have shown that human MSCs are stable<sup>29, 54</sup>. While murine MSCs are more prone to genetic transformation during *in vitro* culture, a recent report supported the safety of MSCs for clinical application on follow up after *in vivo* implantation for orthopedic use<sup>55</sup>. Nonetheless, the importance of stringent monitoring of MSCs, including karyotyping, before application in the clinical setting should not be overlooked.

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