Targeting the Interleukin-8 Signaling Pathway in Colorectal Cancer: A Mini-review

Ishita Aggarwal1*

Colorectal cancer (CRC), commonly called colon cancer, is a cancer of the colon or rectum. Although colon cancer is highly treatable, the National Cancer Institute of Canada (NCIC) acknowledges it as the third most common cancer and the second most common cause of cancer-related deaths among Canadians. Scientific studies have shown that colon cancer is caused, in part, by the overproduction of a molecule called Interleukin-8 (IL-8), which is released from the surface of tumor cells. Once generated, IL-8 binds to specialized proteins, called CXCR2 receptors, on the surface of nearby cancer cells. Binding of IL-8 to CXCR2 produces signals within tumor cells that activate molecules called transcription factors. The activation of various transcription factors, including NF-κB and AP-1, via the Akt and MAPK signaling pathways, ultimately causes the growth and survival of colon cancer cells. Hence, reducing the expression of IL-8 by cancer cells may have therapeutic implications for patients suffering from colon cancer. Although various treatment methods have been developed to inhibit the production of IL-8, most techniques pose a safety risk to patients because they may interact with the human immune system in unpredictable ways. This review suggests that the safest treatment method to target IL-8 is the use of nano-particles, specifically quantum dots (QDs), to transport small interfering RNA (siRNA) into colon cancer cells. Once delivered, siRNA can silence IL-8 expression, reducing the risk of cancerous growth.

INTRODUCTION

Colorectal cancer (CRC) is a cancer of the colon or rectum and is the second most common cause of cancer-related deaths in Canadian men and women (Jemal, Siegel, Xu, & Ward, 2010). Studies have shown that chemokines (proteins secreted by cells) and their receptors are regulators of metastatic diseases, including CRC (Balkwill, 2004; Waugh & Wilson, 2008). By activating intracellular signaling pathways in target cells, chemokines can promote the proliferation and survival of cancer cells within the tumor microenvironment (Balkwill, 2004). Hence, chemokines and their receptors may be important targets for novel drug and intervention therapies (Balkwill, 2004; Waugh & Wilson, 2008).

IL-8, also called CXCL8, belongs to a superfamily of chemically related chemokines that stimulate neutrophil chemotaxis and degranulation. Levels of IL-8 are low in healthy tissues. However, IL-8 is rapidly generated in the presence of pro-inflammatory cytokines, including TNF-α and IL-1β (Hoffmann, Dittrich-Breiholz, Holtmann, & Kracht, 2002). The 77-amino acid protein exerts its biological effects by binding to two chemokine receptors, CXCR1 (IL-8RA) and CXCR2 (IL-8RB), which are members of the seven transmembrane G-protein-coupled receptor (GPCR) family (Raman, Baugher, Thu, & Richmond, 2007; Heidemann, Ogawa, & Dwinell, 2003). Signals are transmitted across the membrane via ligand-induced conformational changes, activating downstream pathways that promote the malignant development of cancer (Waugh & Wilson, 2008; Raman, Baugher, Thu, & Richmond, 2007; Heidemann, Ogawa, & Dwinell, 2003). Although CXCR1 and CXCR2 show considerable structural similarity (Waugh & Wilson, 2008), the proliferation and angiogenesis of human colon cancer cells is likely dependent on IL-8 binding to the latter (Raman, Baugher, Thu, & Richmond, 2007; Hoffmann, Dittrich-Breiholz, Holtmann, & Kracht, 2002).

This review will discuss the importance of two well-characterized signaling pathways that are induced downstream of IL-8 receptors in CRC. Furthermore, it will evaluate current methods of targeting IL-8 and IL-8 receptors to suppress tumor progression and development. Finally, it will offer support for the use of QDs in the treatment of human colon cancer cells.

DISCUSSION

IL-8 signaling pathways

The increased synthesis and secretion of IL-8 from tumor cells and the overexpression of the CXCR2 receptor in endothelial cells and neutrophils indicate that IL-8 and CXCR2 play a crucial role in the development of CRC (Waugh & Wilson, 2008; Raman, Baugher, Thu, & Richmond, 2007). Binding of ligand to CXCR2 stimulates multiple signaling cascades, two of which promote the activation of Akt and mitogen-activated protein kinase (MAPK) (Figure 1), contributing to CRC metastasis (Knall et al., 1996; Knall, Worthen, & Johnson, 1997).

Activation of Akt signaling cascade

Binding of IL-8 to CXCR2 activates intracellular phosphatidylinositol-3 kinase (PI3-K) (Knall, Worthen, & Johnson, 1997). Knall et al. (1997) demonstrated that activation of PI3-K results in the phosphorylation of Akt, a serine/threonine kinase also called Protein Kinase B (PKB). They concluded that the phosphorylation of Akt increases its activity (Knall, Worthen, & Johnson, 1997). Upregulation of Akt has been detected in various forms of cancer, including CRC, and its role in mediating cell survival has been documented in multiple studies (Knall, Worthen, & Johnson, 1997; MacManus et al., 2007). Active Akt

---

1University of Toronto, 27 King’s College Circle, Toronto ON Canada M5S1A1.

*To whom correspondence should be addressed.
promotes cancer development via two pathways. In one cascade, Akt directly stimulates the activity of NF-κB (Huang, DeGuzman, Bucana, & Fidler, 2000; Karin, Cao, Greten, & Li, 2002; Richmond, 2002). Huang et al. (2000) showed that this transcription factor regulates the expression of genes that promote cell proliferation, invasion, and angiogenesis. By transfecting metastatic human melanoma variant cells with an inhibitor of NF-κB, they documented a decrease in tumor growth and lung metastasis in mice (Huang, DeGuzman, Bucana, & Fidler, 2000). In a second cascade, phosphorylated Akt activates mTOR, a transcription factor that ultimately overexpresses various oncogenes, including rS6K and S6, resulting in an increase in protein synthesis and cell proliferation (Waugh & Wilson, 2008; Huang, DeGuzman, Bucana, & Fidler, 2000). As a result, the Akt signaling cascade, induced by IL-8 binding to CXCR2, contributes to the onset of CRC. Thus, targeting IL-8 and CXCR2 may have important therapeutic implications for colon cancer patients.

**Figure 1.** IL-8 signalling pathways in colorectal cancer. Binding of IL-8 to CXCR1 and/or CXCR2 activates heterotrimeric G proteins, which stimulate effector proteins, including phosphatidylinositol-3-kinase. Activation of primary effectors promotes the activation of Akt and/or MAPK signalling pathways, causing protein translation, cell survival, cell proliferation, and/or angiogenesis. Solid lines indicate transcription factors whose activity is upregulated by IL-8 signalling. Dashed lines show pathways through which IL-8 regulates transcription factors. **Image From:** Waugh, D. J., & Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clin Cancer Res.*, 14, 6735-6741.

### Activation of MAPK signaling cascade

IL-8 signaling has been shown to induce the activation of the classic MAPK pathway in CRC. When bound to IL-8, CXCR2 undergoes a conformational change that allows Ras-GTPase to exchange its GDP for GTP, activating the Ras-GTPase (Knall et al., 1996). According to Sparmann et al., Ras-GTP stimulates MAP3K (specifically Raf), a serine-threonine kinase which further activates MAP2K (specifically MEK) (Knall et al., 1996; Sparmann & Bar-Sagi, 2004). MAP2K in turn stimulates MAPK, which activates the transcription factor AP-1 (Knall et al., 1996; Collins, Lee, & Ting, 2000). A study by Collins et al. (2000) demonstrated that AP-1 upregulates the expression of pro-inflammatory cytokines, resulting in cell proliferation, cell survival, and invasion. **Hence,** the MAPK signaling cascade, prompted by IL-8 binding to its receptor, contributes to the onset of CRC. Thus, inhibiting IL-8 signaling may affect the treatment of CRC.

### TARGETING IL-8 AND CXCR2 FOR THERAPEUTIC PURPOSES IN COLORECTAL CANCER

As demonstrated above, the expression of IL-8 and CXCR2 by cancer cells significantly contributes to the tumor microenvironment. Therapies that aim to suppress the IL-8 signaling pathway, which include the use of small molecule antagonists, humanized monoclonal antibodies, small interfering RNA (siRNA) vectors, and nano-particles, can reduce cell proliferation, decreasing the risk of tumorigenicity. As a result, inhibiting the effects of IL-8 signaling may have therapeutic implications for colon cancer patients.

#### Small molecule antagonists/humanized monoclonal antibodies

Currently, small molecule antagonists and humanized monoclonal antibodies are used to attenuate the effects of IL-8 signaling. A study conducted by Ning et al. (2012) found that a small molecule inhibitor of CXCR2, namely SCH-527123, can successfully treat metastatic CRC. SCH-527123 was orally administered to colon cancer mice models, which then exhibited a reduction in tumor volume when compared to control groups (Ning et al., 2012). It was concluded that SCH-527123 can inhibit cell proliferation and can cause programmed cell death in colon cancer cells (Ning et al., 2012). The result was validated in a study conducted by Singh et al. (2009). Additional small molecule inhibitors, including SB 225002, SB 656933, and SCH-479833, have been shown to suppress inflammatory diseases as well (Singh et al., 2009; White et al., 1998). In addition to small molecule antagonists, humanized monoclonal antibodies, such as ABX-IL-8, have been developed to suppress the effects of IL-8 signaling (Mian et al., 2003). A study by Mian et al. (2003) demonstrated that ABX-IL-8 reduces tumor growth and angiogenesis in bladder cancer xenograft models. It is probable that humanized monoclonal antibodies can be used as a treatment for patients with CRC as well. However, a disadvantage of using small molecule inhibitors and humanized monoclonal antibodies to suppress the effects of IL-8 is that they may interact with the immune system via unpredictable and harmful mechanisms (Ning et al., 2012; Mian et al., 2003).

#### siRNA vectors

Knockdown of IL-8, using siRNA vectors, has been shown to
decrease migration and invasion of colon cancer cells. In a study by Cao et al. (2005), downregulation of IL-8 expression was achieved by constructing viral vectors that were used to deliver siRNA to human airway epithelial cells. Similar results have been achieved in various cancers using a non-viral vector-based siRNA delivery approach as well (Chen, Du, Zhang, & Liang, 2005). siRNA-based treatment techniques can be used effectively in low concentrations and can inhibit gene expression with increased specificity (Ali, Urbanati, Raouane, & Massaad-Massade, 2012). Hence, it is likely that siRNA vectors can function to control IL-8 signaling in CRC. However, a drawback of using siRNA vectors to downregulate IL-8 is that they may pose a safety risk to patients. For instance, in rare cases, siRNA vectors can combine with the oncogenes that they are meant to target, upregulating their expression (Ali, Urbanati, Raouane, & Massaad-Massade, 2012).

**Nano-technology/nano-particles (QDs)**

Nano-particles, specifically QDs, may be used to deliver siRNA to colon cancer cells, molecules that can then silence the effects of IL-8 signaling and prevent the development of CRC. Currently, a wide range of tumor suppressors are available to target cancerous cells in the body. However, most tumor suppressors are drug-loaded vesicles that cannot specifically target a tumor site (Xiao, Forry, Gao, Holbrook, Telfor et al., 2010). Recognized for their photophysical properties, QDs are non-hazardous vectors for gene delivery (Derfus, Chen, Min, Ruoslahti, & Bhattacharya, 2007) and may be used to overcome the non-specificity of most tumor suppressors currently on the market (Xiao, Forry, Gao, Holbrook, Telfor et al., 2010). Wang et al. (2007) demonstrated that QD-mediated binding of target cells is highly specific. They found that delivery of QDs is dependent on expression levels of epidermal growth factor receptor (EGFR), a molecule that is correlated with the severity of cancerous diseases (Wang, Yong, Sun, Vernier, Koeffler et al., 2007). When transfected into diseased tissue, QDs reached EGFR-expressing cell lines rapidly, indicating QD target specificity (Wang, Yong, Sun, Vernier, Koeffler et al., 2007). Furthermore, QDs overcome the limitations of siRNA vectors by allowing for cellular penetration, endosomal release, unpackaging, intracellular transport, and protection from degradation (Li et al., 2011). They can be excreted from the body by the liver, lowering the risk of cytotoxicity for patients (Derfus, Chen, Min, Ruoslahti, & Bhattacharya, 2007; Li et al., 2011). Hence, the use of QDs presents a novel approach to battling colon cancer.

**CONCLUSION**

In conclusion, multiple studies have demonstrated that IL-8 is a pro-inflammatory chemokine that activates numerous signaling cascades in tumor cells, causing the proliferation and survival of colon cancer. Consequently, the IL-8 signaling pathway may be an important therapeutic target in patients suffering from CRC. To date, small molecule antagonists, humanized monoclonal antibodies, and siRNA vectors have been primarily used to weaken the effects of IL-8 on the tumor microenvironment. Targeting IL-8 and CXCR2 in CRC may prove more effective if nanotechnology were used in conjunction with siRNA silencing strategies. Regardless, mechanisms of IL-8 signaling in CRC and potential treatment methods to control overexpression of the chemokine need to be furthermore investigated.

**REFERENCES**


based siRNA delivery for HPV18 E6 gene silence and intracellular imaging. *Biomaterials, 32*, 7978-7987.


