Novel Control Of Cell Migration In Cancer

Popeye domain-containing (POPDC) proteins are effector proteins that bind to cAMP to create a second messenger response that can influence the behaviour of cancer cells (Amunjela & Tucker, 2016). There are three different genes POPDC1, POPDC2 and POPDC3 that encode these proteins, however only POPDC1 and POPDC3 have been related to cancer cell behaviour. POPDC1 and POPDC3 are organised in tandem on chromosome 6q21 and POPDC2 can be found on chromosome 3 (Andree et al., 2000). All three of these proteins are found within embryonic epithelium, the heart and skeletal muscle (Kim et al., 2010). POPDC proteins are transmembrane proteins that consist of an extracellular amino terminus, three transmembrane domains and a cytoplasmic Popeye domain (Amunjela & Tucker, 2016).The Popeye domain is where cAMP binding occurs. cAMP is a key second messenger that regulates many physiologically crucial processes such as cell growth, gene transcription and expression (Yan et al., 2016). This signal transduction cascade begins when a specific ligand binds to a G-protein-coupled receptor (GPCR). This causes a conformational change in the protein and a G?s stimulatory subunit is released and activates the enzyme adenylyl cyclase which converts ATP into cAMP (Amunjela & Tucker, 2016). There are three key effector molecules that bind to cAMP and signal in cancer cells known as protein kinase A (PKA), Epac proteins and POPDC proteins (Amunjela & Tucker, 2016).

These signalling cascades have substantial roles in modulating the proliferation and migration of cancer cells and are therefore, especially POPDC proteins, targeted as potential therapies to treat cancer as their domains can be targeted specifically to prevent growth and spreading of these cancer cells throughout the body (Amunjela & Tucker, 2016). Here, the role of POPDC proteins in cancer progression, their interaction with other genes such as GEFT to regulate signalling cascades occurring in cancer cells, and how this makes them targets for cancer therapies is discussed. Several studies have demonstrated that POPDC1 and POPDC1 expression is downregulated in cancer cells. One of these studies by Kim et al. (2010) confirms the downregulation of POPDC proteins in gastric cancer cells. A real-time quantitative PCR (qRT-PCR) was used to analyse 96-paired gastric tumours and their nearby normal tissues. The expression of POPDC1 was downregulated in 69% (66 of 96) of the gastric tumours and POPDC3 expression was downregulated in 87% (83 of 96) of the gastric tumours. However, POPDC2 expression was only downregulated in 24% (23 of 96) of the gastric cell tumours. This determines that POPDC2 is not associated with cancer development as significantly as POPDC1 and POPDC3 proteins.

Deng et al., (2012) conducted an immunohistochemical analysis to discover the amount of POPDC3 expression in 306 gastric cancer tissues and 84 normal tissues and related this to prognosis of stage 1 and stage 2 cancer patients. Elevated levels of POPDC3 expression were found in 72 of 84 normal gastric tissues whereas low expression of POPDC3 was found in 228 of 306 gastric cancer tissues. This suggests that POPDC3 expression has a role in downregulating cancer progression. This is confirmed by Deng et al., (2010) as the prognosis of cancer patients that present with higher POPDC3 expression in their cancerous cells is significantly better than in patients with lower POPDC3 expression.

To determine whether POPDC3 downregulation is involved in migration in gastric cancer cells,

Kim et al., (2010) studied how shRNA-mediated deletion of POPDC3 expression in SNU-216 cells affected the levels of migration. POPDC3 expression was downregulated by POPDC3-sh#1 and -sh#2. POPDC3 was then subjected to a cell migration assay. The cells treated with EGF showed an increase in cell migration in comparison to the controls. In addition, POPDC3-sh#1 or -sh#2 containing cells showed a substantial increase in cell migration in comparison to control cells, suggesting that the downregulation of POPDC proteins in gastric cancer cells induces cell migration and invasion in gastric cancer cells. DNA methylation is frequently used to silence gene transcription by adding methyl groups to DNA and modifying histones by adding a histone deacetylase to the DNA (Nan et al., 1998). DNA methylation and histone deacetylation play a role in POPDC1 and POPDC3 silencing in gastric cancer cells (Kim et al., 2010).

A study by Kim et al., (2010) confirms DNA methylation in POPDC1 and POPDC3 genes by using pyrosequencing to discover the amount of methylation in POPDC1 and POPDC3 genes in 76-paired gastric tumour and normal tissues. The mean methylation for POPDC1 was higher in tumour tissues (18.0 ± 9.6%) in comparison to normal tissues (9.4 ± 7.9%). The mean methylation for POPDC3 was also higher in tumour tissues (26.9 ± 21.6%) in comparison to normal tissues (13.2 ± 8.9%), suggesting that DNA methylation plays a role in silencing POPDC1 and POPDC3 proteins in gastric cancer cells. Kim et al., (2010) then set out to determine whether the treatment of DNA methylation inhibitor 5-aza-dC or histone deacetylase inhibitor TSA would induce the expression of POPDC1 and POPDC3 in gastric cancer cells. As seen in Figure 6, a combination of both inhibitors increased expression of POPDC1 and POPDC3, suggesting that these inhibitors are key to initiating POPDC protein expression and therefore, preventing cancer progression.An important discovery that has given insight into how POPDC proteins regulate cancer cell behaviour is through the interaction with guanine exchange factor T (GEFT). This was experimented using a yeast two-hybrid assay in embryonic mouse heart cells (Guo et al., 2003).

GEFT is a guanine exchange factor that activates Rho GTPases such as Ras-related C3 botulinum toxin substrate (Rac1) and cell division control protein 42 (Cdc42). Rac1 and Cdc42 are known to control cellular behaviours such as cell proliferation, migration, cell-cell adhesion, and gene expression (Bishop & Hall, 2000). In relation to cancer, these proteins are excessively synthesised in many carcinogenic tumours, suggesting that they play a role in cancer progression.

To determine whether POPD1 expression changes the activity levels of Rac1 and Cdc42 GTPases, a methodology known as a PAK-21 pulldown was used to determine GTPase activity upon binding to POPDC1 (Smith et al., 2008). The carboxyl terminus of the POPDC1 protein in mouse cells was transfected with pEGFP-mBvesCT or pEGFP-C3 (control). Transfection of mBves-CT negatively regulates the activity of Rac1 and Cdc42 when it interacts with GEFT, resulting in less active levels of Rac1 and Cdc42 and therefore, a reduction in cancer development (Smith et al., 2008). It is evident that the downregulation or deletion of POPDC proteins play a significant role in cancer progression, specifically in gastric cancer cells. Among the three POPDC genes, POPDC1 and POPDC3 expression are substantially reduced in gastric cancer cells whilst POPDC2 expression remains unchanged. A study by Kim et al., (2010) confirmed that the downregulation or silencing of POPDC3 and POPDC1 induced the spread and growth of gastric cancer cells, ultimately confirming that the deletion or suppression of these proteins are associated with a poor prognosis for cancer patients as it can lead to proliferation, migration, and invasion of cancer cells (Amunjela & Tucker, 2016).

Downregulation of these proteins can occur from hypermethylation and histone deacetylation, which epigenetically suppresses POPDC gene expression (Kim et al., 2010). In a study by Kim et al., (2010) POPDC1 and POPDC3 genes had significantly higher amounts of hypermethylation in gastric tumour tissues in comparison to normal gastric tissues, indicating that hypermethylation of both genes is an important event in gastric carcinogenesis. However, treating these hypermethylated genes with DNA methylation inhibitor 5-aza-dC and a histone acetylase inhibitor TSA induced POPDC gene expression (Kim et al., 2010). This provides a promising theory that genes pharmacologically treated with these inhibitors could be an effective way of inhibiting cancer progression through increasing their POPDC gene production.

The discovery of the interaction between POPDC1 and guanine exchange factor T (GEFT) has given insight into the effects of how POPDC proteins can reduce cancer progression from growing and spreading throughout the body. GEFT activates Rho GTPases Rac1 and Cdc42 which are drastically overexpressed in human tumours as they are responsible for regulating cell-cell bonding, proliferation, and migration (Bishop & Hall, 2000). When POPDC1 encounters GEFT in cancer cells, Smith et al., (2008) discovered that it negatively regulates the active levels of Rac1 and Cdc42 in cancer cells. This confirms that POPDC proteins play a significant role in inhibiting cancer progression throughout the body by preventing cancer cells from multiplying and spreading to different tissues through upregulation of Rac1 and Cdc42.

In summary, it is clear that the relationship discovered between cancer development through proliferation and migration of cancer cells in humans and the downregulation and deletion of POPDC proteins and genes demonstrates that POPDC proteins could be a promising and effective pharmacological treatment in combating cancer progression and it is therefore crucial to investigate the structure and functions of POPDC proteins further to improve the survival rate of many cancer patients.