The role of Popeye domain-containing protein 1 (POPDC1) in the progression of the malignant phenotype

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The Popeye domain-containing protein 1 (POPDC1), a tight junction-associated transmembrane protein with a unique binding site for cAMP, has been shown to act as a tumour suppressor in cancer cells. Through interaction with many downstream effectors and signalling pathways, POPDC1 promotes cell adhesion and inhibits uncontrolled cell proliferation, epithelial-to-mesenchymal transition and metastasis. However, POPDC1 expression is down-regulated in many types of cancer, thereby reducing its tumour-suppressive actions. This review discusses the role of POPDC1 in the progression of the malignant phenotype and highlights the broad range of benefits POPDC1 stabilisation may achieve therapeutically. Cancer stem cells (CSCs) are a key hallmark of malignancies and commonly promote treatment resistance. This article provides a comprehensive overview of CSC signalling mechanisms, many of which have been shown to be regulated by POPDC1 in other cell types, thus suggesting an additional therapeutic benefit for POPDC1-stabilising anti-cancer drugs.

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KEYWORDS
CAMP, cancer, cancer stem cells, epithelial-to-mesenchymal transition, metastasis, POPDC1, proliferation

1  |  INTRODUCTION

The Popeye domain-containing protein 1 (POPDC1) was discovered in 1999, and research over the last 20 years has shown it to be involved in the progression of malignancies. Initially discovered in cardiac tissue, POPDC1 (also known as blood vessel epicardial substance [BVES]) has a wide tissue distribution and is found in smooth and skeletal muscle, the brain, gastrointestinal (GI) tract,
and liver (Han et al., 2019). It is also present in many cancers, such as breast cancer (BC), gastric cancer (GC), colorectal cancer (CRC), non-small cell lung carcinoma (NSCLC), hepatocellular carcinoma (HCC), and uveal melanoma (UM) (Han et al., 2019). Although POPDC1 proteins regulate signalling pathways in cardiac and skeletal muscle tissue (Brand, 2018), the focus of this review will be their role as tumour suppressors. Dysregulation of POPDC1 expression in cancer cells may offer an interesting and novel therapeutic target in the treatment of cancer and will be discussed in the following article.

1.1 The structure of the POPDC1 protein

Popeye domain-containing proteins exist in three isoforms: POPDC1, POPDC2, and POPDC3. Structurally, these isoforms differ in their amino acid chain length: 360, 364, and 291 residues, respectively (Brand & Schindler, 2017). It has been suggested that these three subtypes and their splice variants exist to facilitate tissue-specific target interactions, as a result of truncated binding regions (Benesh et al., 2013). In addition to the variations in amino acid chain length, the POPDC2 and POPDC3 isoforms differ in their intracellular phosphorylation sites (Brand & Schindler, 2017) and have only one extra-cellular glycosylation site at aa4 (UniProt Consortium, 2019, available at: https://www.uniprot.org/uniprot/Q9HBU9 and https://www.uniprot.org/uniprot/Q9HBV1). Interestingly, whereas the POPDC2 gene is located on 3q13.33, the POPDC1 and POPDC3 genes are found on the same chromosome (6q21), which suggests that the POPDC3 gene may have arisen as a result of a tandem duplication of the POPDC1 gene (Schindler et al., 2012).

### Table 1: The role of POPDC2 and POPDC3 in disease

<table>
<thead>
<tr>
<th>POPDC2</th>
<th>POPDC3</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>Under-expressed causing decreased conduction and AV block in cardiac muscle only. Associated with W188X mutation</td>
<td>Amunjela et al., 2019; Brand, 2019</td>
</tr>
<tr>
<td>Limb girdle muscular dystrophy (LGMD)</td>
<td>Mutant (L155H, L217F, R261Q) POPDC3 expression leads to skeletal muscular dystrophy</td>
<td>Vissing et al., 2019</td>
</tr>
<tr>
<td>Ductal breast carcinoma (especially HER2+ subtype)</td>
<td>Overexpressed at all clinical stages. Possibly implicated in cancer initiation and sustenance</td>
<td>Amunjela &amp; Tucker, 2017a</td>
</tr>
<tr>
<td>Head and neck squamous cell carcinoma (HNSCC)</td>
<td>Overexpression correlates with low patient survival. Potential biomarker for radiotherapy resistance</td>
<td>He et al., 2019</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Under-expression due to promoter hypermethylation. Lower POPDC3 levels correlate with increased depth of invasion and metastasis</td>
<td>Kim et al., 2010; Luo et al., 2012</td>
</tr>
<tr>
<td>Oesophageal and lung cancer</td>
<td>Overexpression of POPDC3 correlates with greater radiotherapy resistance</td>
<td>He et al., 2019</td>
</tr>
</tbody>
</table>

Note: Expression of POPDC2 and POPDC3 varies between tissue type and across various cancer types. Dysregulation of POPDC2 is mainly observed in cardiovascular disease and breast cancer. POPDC3 mutations are implicated in limb girdle muscular dystrophy and has been shown to have both tumour-suppressive and oncogenic roles in different malignancies.
different splice variants of the POPDC1 protein. The level of glycosylation seems to differ between tissue types, with the extent of glycosylation shown to be greater in skeletal muscle and brain tissue than cardiac tissue, which may be of functional relevance (Brand, 2019; Vasavada et al., 2004). The extent of POPDC1 glycosylation in cancer cells and the functional relevance of this has yet to be investigated.

This extracellular sequence is followed by three transmembrane domains and a larger, intracellular carboxy-terminal domain (CTD) (aa114–aa360). The intracellular CTD contains the highly conserved POPEYE domain (aa172–aa276) with a unique cAMP binding site. Other binding sites exist for TREK1, VAMP3, Bnip3, LRPI, ZO-1, GEFH, CAV-3 (aa242–aa246), GEFH (aa250–aa300), and PR61x (aa330–aa345). A disulfide bridge forms between intracellular cysteine residues with lysine residues (aa272/aa273) critically required for this dimerisation, which promotes membrane stabilisation.

In vitro studies by Osler et al. (2005) using epicardial mesothelial cells showed that before cell–cell contact was established, POPDC1 was localised in the cytoplasm. Once contact between neighbouring cells formed, POPDC1 inserted itself into the cell membrane and co-localised with other junctional proteins, resulting in cell adhesion (Osler et al., 2005). Furthermore, POPDC1 proteins that are no longer inserted into the cell membrane (e.g., in BC or HCC cells) have been shown to accumulate intracellularly (Amunjela et al., 2019; Han et al., 2015; Russ et al., 2011), but it remains unclear whether this accumulation is a pathway to POPDC1 degradation or a progressive storage mechanism. The potential effect of nuclear POPDC1 on gene transcription has also yet to be elucidated (Amunjela et al., 2019).

1.2 | Hallmarks of cancer

Cancer, the inappropriate and uncontrolled growth of cells, which can lead to the formation of malignant tumours, is one of the leading
causes of death worldwide. Over 20 years ago, Hanahan and Weinberg defined six key hallmarks, which characterise the malignant phenotype of this disease. These include self-sufficient growth signalling (cancer cells do not require endogenous signals but generate their own growth factors to stimulate proliferation), acquired resistance to growth-inhibiting signals, evasion of apoptosis, replicative immortality (by reactivating telomerase reverse transcriptase [TERT] to prevent telomere shortening), sustained angiogenesis (to maintain oxygen and nutrient supply allowing tumour growth), and tissue invasion and metastasis (Hanahan & Weinberg, 2000). A decade later, the authors extended their list of hallmarks, adding four enabling characteristics of cancer, which are not unique to cancer cells but help drive disease progression. Genome instability and an inflammatory tumour environment have been shown to promote malignant growth, whilst altered cellular energetics and immune system evasion (due to surface antigen loss and cancer stem cell [CSC] formation) further enable cancer progression (Hanahan & Weinberg, 2011). The most relevant hallmarks in the context of this review are uncontrolled cell proliferation, loss of cell adhesion, and metastasis, as well as the presence of CSCs, which drive malignancy, treatment resistance, and cancer recurrence (Du et al., 2019). The treatment options for cancer are based on inhibiting these processes; however, current therapies show varying degrees of success. As the POPDC1 protein has been shown to play a role in cancer cell proliferation, loss of adhesion, and metastasis (Figure 2), this protein presents an exciting target for developing novel anti-cancer drugs.

### TABLE 2 POPDC1 downstream targets

<table>
<thead>
<tr>
<th>Protein</th>
<th>Interacting sequence of POPDC1</th>
<th>Tissue location of POPDC1 interaction</th>
<th>Suggested role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRED-1</td>
<td>Unknown sequence on CTD</td>
<td>Cardiac myocytes</td>
<td>Interaction with POPDC1 enhances current flow in cardiac myocytes</td>
<td>Brand, 2019; Han et al., 2019</td>
</tr>
<tr>
<td>CAV-3</td>
<td>aa242-aa266</td>
<td>Skeletal muscle sarcolemma, cardiac myocyte transverse tubules</td>
<td>POPDC1 ensures structural integrity and function of Cav-3</td>
<td>Brand, 2019; Han et al., 2019</td>
</tr>
<tr>
<td>VAMP3</td>
<td>CTD sequence after aa118</td>
<td>MDCK cells, adult cardiac and skeletal muscle</td>
<td>POPDC1 interaction ensures adequate recycling of β1 integrins. Loss of this interaction increases migration</td>
<td>Hager et al., 2010</td>
</tr>
<tr>
<td>GEFH</td>
<td>aa250-aa300</td>
<td>Human corneal epithelia, murine NIH T3T cells</td>
<td>Retention of GEF in membrane, preventing Rac1/Cdc42/RhoA activation promoting TJ formation</td>
<td>Russ et al., 2010; Smith et al., 2008</td>
</tr>
<tr>
<td>GEFH</td>
<td>Unknown sequence on CTD</td>
<td>Human corneal epithelia</td>
<td>POPDC1 sequesters GEFH to cell membrane to prevent RhoA signalling</td>
<td>Parang et al., 2018; Russ et al., 2011</td>
</tr>
<tr>
<td>ZO-1</td>
<td>Unknown sequence on CTD</td>
<td>Trabecular meshwork cells, HCE, uveal melanoma</td>
<td>POPDC1/ZO-1 interaction prevents ZONAB-induced entry to cell cycle and translation of proliferative genes</td>
<td>Amunjela et al., 2019; Jayagopal et al., 2011; Russ et al., 2010, 2011</td>
</tr>
<tr>
<td>Occludin</td>
<td>Unknown sequence on CTD</td>
<td>HCE, uveal melanoma</td>
<td>Maintenance of tight junction formation</td>
<td>Amunjela et al., 2019; Jayagopal et al., 2011; Russ et al., 2010, 2011</td>
</tr>
<tr>
<td>Bnip3</td>
<td>Unknown sequence on CTD</td>
<td>Cardiac myocytes</td>
<td>POPDC1 suppresses Bnip3-induced apoptosis</td>
<td>Kliminski et al., 2017</td>
</tr>
<tr>
<td>LRP6 (Wnt/β-catenin pathway)</td>
<td>Unknown sequence on CTD</td>
<td>HEK293 cells, human colonoids, murine adenoma tumouroids</td>
<td>Prevention of β-catenin activation by inhibition of LRP6</td>
<td>Thompson et al., 2019</td>
</tr>
<tr>
<td>PR61α (c-Myc pathway)</td>
<td>aa330-AA345</td>
<td>Murine colitis-associated cancer cells</td>
<td>Promotes c-Myc ubiquitination/degradation</td>
<td>Parang et al., 2017</td>
</tr>
</tbody>
</table>

Note: The POPDC1 protein interacts with many downstream including TREK1, CAV-3, VAMP3, GEFH, GEFH, ZO-1, occludin, Bnip3, LRP6, and PR61α. This interaction has mainly been shown in cardiac and skeletal muscle cells; however, an increasing body of evidence is emerging that demonstrates POPDC1 interaction with these targets in cancer cells.

Abbreviations: CTD, C-terminal domain; HCE, human corneal epithelial cell; HEK293, HEK cells.
1.3 | Signalling mechanisms and downstream targets of POPDC1

The POPDC protein family is one of the five downstream targets of cAMP, the other four being PKA, exchange protein directly activated by cAMP (Epac), hyperpolarisation-activated cyclic nucleotide-gated channel (HCN), and cyclic nucleotide receptor involved in sperm function (CRIS) (Schindler & Brand, 2016). cAMP binds to the PBC of the POPEYE domain (discussed above) and leads to increased protein expression, stabilisation, and activation (Amunjela et al., 2019). In cancer cells, increased cellular levels of cAMP correlate with increased apoptosis and reduced proliferation, invasion, and metastasis. As other downstream effectors of cAMP such as PKA and Epac increase these effects, this suggests that downstream pro-apoptotic effects of increased cAMP in cancer cells could, at least in part, be mediated by the actions of POPDC1 proteins (Amunjela et al., 2019).

POPDC1 also influences other signalling pathways, such as the Wnt pathway, and transcription factors, such as c-Myc, and to interact with proteins including CAV-3, TREK1 (two-pore domain potassium channel), TJ-associated proteins (e.g., ZO-1 and occludin), guanine nucleotide exchange factors (GEFT and GEFH) (Parang et al., 2018; Smith et al., 2008), and the vesicular transport protein VAMP3 (Han et al., 2019) (Figure 2 and Table 2). Although the interactions of POPDC1 with TREK1, CAV-3 and Bcl-2 and adenovirus E1B 19-kDa interacting protein 3 (Bnip3) has thus far mainly been demonstrated in cardiac and skeletal muscle function (Brand, 2019), recent studies have found that TREK1 is overexpressed in prostate cancer (PC) cells, CAV-3 is up-regulated in anaplastic thyroid carcinoma (Han et al., 2019), and Bnip3 levels are increased in BC and NSCLC (Lee & Paik, 2006). However, the presence of POPDC1 and interaction with these proteins in cancer cells has yet to be established. The known effects of POPDC1 on downstream targets in cancer cells will be discussed in this review.

1.4 | Down-regulation of POPDC1 in cancer cells

The down-regulation of POPDC1 proteins has been demonstrated in a large range of cancer cell types. Loss of POPDC1 expression has been proposed to be initiated by four main mechanisms. Firstly, hypermethylation of the cytosine–phosphate–guanine islands of the POPDC1 gene promoter has been demonstrated in a wide range of cancers (Table 3) and has hence been suggested as a biomarker for early detection of cancer (Parang et al., 2018). Secondly, the under-expression of microRNA (miRNA)-122 (Wang et al., 2014) and, thirdly, overexpression of netrin-1 (Han et al., 2015) are known to suppress POPDC1 expression. Finally, increased EGFR stimulation leads to a significant suppression of POPDC1 activity, most likely as a result of phosphorylation of the POPDC1 CTD (Amunjela & Tucker, 2017a).
The mechanisms implicated in different cancers are shown in Table 3. The four main mechanisms of POPDC1 down-regulation include promoter hypermethylation, under-expression of miRNA-122 reducing POPDC1 gene transcription, overexpression of netrin-1 leading to inhibited POPDC1 expression, and EGFR activation, which phosphorylates and inactivates POPDC1. These mechanisms have been observed in many different cancer types including HCC, CRC, BC, PC, NSCLC, HNSCC, and glioma. The most commonly identified mechanism of POPDC1 down-regulation is promoter hypermethylation. Abbreviations: BC, breast cancer; CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; PC, prostate cancer.

Note: The four main mechanisms of POPDC1 down-regulation include promoter hypermethylation, under-expression of miRNA-122 reducing POPDC1 gene transcription, overexpression of netrin-1 leading to inhibited POPDC1 expression, and EGFR activation, which phosphorylates and inactivates POPDC1. These mechanisms have been observed in many different cancer types including HCC, CRC, BC, PC, NSCLC, HNSCC, and glioma. The most commonly identified mechanism of POPDC1 down-regulation is promoter hypermethylation. Abbreviations: BC, breast cancer; CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; PC, prostate cancer.

2.1 | Interaction with ZO-1 and ZONAB

POPDC1 interacts with ZO-1 (aka TJ protein 1, TJP1) to maintain cell adhesion and TJ-associated signalling and to prevent migration (Russ et al., 2011). This interaction allows ZO-1 to recruit ZONAB/DbpA to the TJ and prevent its nuclear localisation (Amunjela et al., 2019). If POPDC1 protein expression is lost in cancer cells, ZONAB/DbpA can translocate to the nucleus. ZONAB/DbpA is a Y-box transcription factor, and via interaction with cell division kinase 4 (CDK4), it increases transcription of HER2, cyclin D1 and PCNA (Amunjela et al., 2019; Jayagopal et al., 2011), allowing entry into the cell cycle and progressive cell proliferation. When looking at the interaction of POPDC1 with these proteins in cancer cells, ZO-1 and ZONAB interaction has been demonstrated in UM (Jayagopal et al., 2011), and a correlation between POPDC1 and ZO-1 loss has been observed in HCC (Han et al., 2019). The POPDC1/ZO-1 interaction in other cancer types requires further investigations.

2.2 | Interaction with GEFT and GEFH

Rac/Cdc42-specific exchange factor, a guanine nucleotide exchange factor more commonly referred to as GEFT, can interact (via aa300–aa450) with POPDC1 via its C-terminal residues aa250–aa300 (Smith et al., 2008). GEFT preferentially binds to and increases Rac1 and Cdc42 (cell division control protein 42) activity, which promote cell proliferation. In addition, activation of Rac1 and Cdc42 disrupts cell adhesion and promotes cell migration, which is discussed later in this review. POPDC1 negatively regulates GEFT (Smith et al., 2008) and thereby reduces Rac1 and Cdc42 activity, supporting its role as a tumour suppressor. High GEFT activity has been demonstrated in brain tumours (Guo et al., 2003), but the GEFT/POPDC1 interaction has so far only been demonstrated in fibroblasts (NIH T3T cells) (Smith et al., 2008) and human corneal epithelia, where POPDC1 interacting with two different GEFs, GEFT and GEFH, seems to promote TJ formation by reducing RhoA activity (Russ et al., 2010; Russ et al., 2011). When epithelial cell confluence has been established through formation of desmosomal, tight and adherens junctions, the activation of RhoA signalling that stimulates further cell proliferation is inhibited by GEFT and cingulin (TJ-associated protein) (Parang et al., 2018; Russ et al., 2011). Hence, loss of TJ formation in cancer cells that under-express POPDC1 could re-establish RhoA signalling and induce cancer cell proliferation.

2.3 | c-Myc pathway

c-Myc is a pro-oncogenic transcription factor commonly overexpressed in cancer cells. Active c-Myc plays an important role in the transcription of proliferative genes, cell cycle control, cell differentiation, and the epithelial-to-mesenchymal transition (EMT) (Kallkat et al., 2017; Parang et al., 2017; Swan et al., 2019). Phosphorylation of the S62 residue of c-Myc by MAPK keeps it in an inactive state and...
thereby maintains a steady c-Myc concentration in the cell. Further phosphorylation of the T58 residue by glycogen synthase kinase 3β (GSK3β) allows subsequent dephosphorylation of the S62 residue by the PR61α subunit of serine–threonine protein phosphatase 2A (PP2A). PP2A is a heterotrimeric protein made up of a regulatory, structural, and catalytic subunit. Once the S62 residue has been dephosphorylated, c-Myc is ubiquitinated and targeted for proteasomal degradation (Seshacharyulu et al., 2013; Yeh et al., 2004).

POPDC1 increases the negative regulation of c-Myc. It interacts with both c-Myc and the PR61α (aka PPP2R5A) subunit of PP2A via a 15-amino acid sequence on its C-terminal end (aa330–aa345), directing it towards the dual-phosphorylated S62/T58 c-Myc and promoting S62 dephosphorylation (Parang et al., 2017), thus increasing degradation of pro-mitotic c-Myc. Loss of POPDC1 in cancer decreased T58 phosphorylation, reduced c-Myc ubiquitination, and increased active c-Myc (Parang et al., 2017), ultimately resulting in increased proliferative signalling.

The c-Myc pathway has long been recognised as a promising anti-cancer drug target, especially as the Myc oncogene is involved in driving many hallmarks of cancer (proliferation, suppressed apoptosis, and altered cell metabolism) and that it is dysregulated in over 50% of all cancers (Chen et al., 2018). However, targeting c-Myc specifically has proven difficult due to its nuclear localisation as a transcription factor. Consequently, other parts of the c-Myc pathway must be investigated for drug development (Chen et al., 2018). As described above, PP2A is crucial for c-Myc ubiquitination and subsequent degradation, and various approaches to increase PP2A activity are currently being investigated for therapeutic use (Remmerie & Janssens, 2019). First data have suggested that POPDC1 interacts with the PR61α subunit of PP2A to increase c-Myc inactivation. Therefore, we hypothesise that stabilising POPDC1/PP2A interactions in cancer cells may be a novel therapeutic approach for promoting c-Myc degradation and reducing its effects on malignant progression.

2.4 | Wnt/β-catenin pathway

The Wnt/β-catenin pathway is a major cell signalling pathway when mutations in pathway components have been linked to the progression of several cancer types, including both solid tumours and blood cancers (Martin-Orozco et al., 2019). The Wnt signalling pathway can be activated in a β-catenin-dependent (canonical pathway) or independent (non-canonical pathway) manner. Usually, in the absence of a Wnt ligand, β-catenin is phosphorylated and maintained in an inactive state by protein kinases GSK3β and casein kinase 1α (CK1α), both part of the axin2 complex. Another enzyme in this complex, β-TrCP, ubiquitinates β-catenin targeting it for proteasomal degradation (Martin-Orozco et al., 2019; Zhan et al., 2017).

Wnt ligands (e.g., Wnt3a or Wnt1) bind and activate the frizzled (Fzd) protein and the neighbouring co-receptor lipoprotein receptor-related protein 6 (LRP6). The LRP6 receptor is in turn phosphorylated by GSK3β and CK1α leading to dishevelled (Dvl) polymerisation. This inactivates the axin2 complex, thus preventing β-catenin inactivation (Butti et al., 2019). β-Catenin can then translocate to the nucleus, where it binds to lymphoid and T cell-enhancing factors (LEF/TEF) and other transcription factors (e.g., cyclin D1), leading to the transcription of proliferative genes (Amunjela et al., 2019; Yu et al., 2012). It also triggers transcription of genes involved in metastasis, which will be discussed later. The activation of the non-canonical pathway and its involvement in cancer cell metastasis will also be examined within this review.

Williams et al. (2011) produced the first results suggesting that POPDC1 was implicated in the regulation of canonical Wnt signalling. It has been since suggested that POPDC1 may exert this role by interacting with the co-receptor of the canonical pathway, LRP6 (Thompson et al., 2019). POPDC1 binding to the LRP6 receptor, which occurs through the intracellular/transmembrane part of LRP6, keeps β-catenin in its inactive state, preventing activation of its downstream targets. The loss of POPDC1 protein expression in cancer cells (see Section 1.4) has been shown to enhance Wnt signalling (Parang et al., 2017). Loss of POPDC1 leads to LRP6 phosphorylation (Ser1490) and recruitment of axin2 to the cell membrane, stabilising β-catenin. This results in increased β-catenin signalling and leads to cellular changes that promote proliferation and metastasis (Thompson et al., 2019). Hence, stabilising the POPDC1 protein to maintain LRP6 interaction may be an effective method of reducing proliferation of cancer cells.

The Wnt signalling pathway is further regulated by PP2A (Thompson & Williams, 2018). While this regulation seems to occur in both positive and negative manner, only the negative regulation of Wnt signalling is mediated through the PR61α subunit. This subunit promotes β-catenin degradation through an APC-dependent signalling complex (Thompson & Williams, 2018). As previously discussed in Section 2.3, POPDC1 interacts with this specific subunit of the PP2A protein and promotes c-Myc degradation. It would be interesting to further investigate and confirm if POPDC1/PR61α interaction has a role in also negatively regulating Wnt signalling.

Pharmacologically targeting this pathway may bring various unwanted side effects due to the ubiquitous nature of the signalling pathway (Raisch et al., 2019). Hence, the LRP6 co-receptor (frequently overexpressed in CRC, hepatic cancer, BC, and pancreatic ductal adenocarcinoma and associated with increased β-catenin signalling, poor prognosis, and chemoresistance) has been explored as a novel, more specific therapeutic target to inhibit the Wnt pathway. Thus far, only the extracellular binding sites of LRP6 have been targeted, by inhibiting Wnt secretion to reduce receptor activation (using porcupine inhibitors), by increasing Dickkopf-related protein 3 concentration (an LRP5/6 antagonist), or by using monoclonal antibodies that prevent Wnt binding to the extracellular binding sites of LRP6 (Raisch et al., 2019). These approaches each come with limitations relating to ligand and/or receptor specificity. Therefore, decreasing LRP6 activation intracellularly through stabilisation of POPDC1 and allowing interaction with the intracellular portion of LRP6 to prevent β-catenin activation could be a novel, specific approach in drug
development to dampen down Wnt signalling and reduce cancer cell proliferation.

### 2.5 | Bnip3

Bnip3 is a mitochondrial, membrane-bound protein that is part of the Bcl-2 family. The Bcl-2 protein family balances cell proliferation and apoptosis, a balance often dysregulated in cancer cells (Hanahan & Weinberg, 2011). Once activated, Bnip3 mediates opening of mitochondrial permeability transition pores and interacts with BAX/BAK proteins to induce apoptosis. Marked increases in Bnip3 expression in hypoxic areas of solid tumours are found in BC and NSCLC but have been shown to be suppressed in other cancer types (especially pancreatic cancer [PAC], CRC, and GC) to prevent apoptosis (Lee & Paik, 2006). POPDC1 suppressed Bnip3 activity in cardiac myocytes, and loss of POPDC1 resulted in Bnip3 up-regulation and increased apoptosis (Kliminski et al., 2017). Further, reduced POPDC1 expression also reduced expression of Bnip3. However, the role of the POPDC1/Bnip3 interaction in cancer cells remains to be investigated.

### 2.6 | Outlook—Inhibiting cancer cell proliferation by stabilising POPDC1

From the data explored above, it is evident that increasing expression or stabilising existing POPDC1 protein in cancer cells could prevent aberrant proliferation through several mechanisms. Firstly, cell proliferation could be inhibited by stabilising POPDC1-mediated cell adhesion. This could maintain POPDC1 interaction with TJ proteins such as ZO-1 and occludin and prevent activation of ZONAB and RhôA and subsequent transcription of proliferative genes. TJ loss has been established in a myriad of cancers (Salvador et al., 2016) and leads to the activation of many downstream signalling pathways (Bhat et al., 2019). Although this makes the TJ an interesting target in cancer treatment (Shah, 2012), a current lack of understanding of how different TJ components are involved in regulating these pathways in cancer poses a barrier to investigating TJ proteins specifically as therapeutic targets (Bhat et al., 2019).

Mechanisms by which the GEFT pathway could be targeted for anti-cancer drug development are not only apparent in cell proliferation but are also strongly implicated in cancer metastasis. Novel approaches to targeting this pathway and potential roles for POPDC1 stabilisation are discussed in the respective sections below.

Finally, the possibilities describing how POPDC1 stabilisation may lead to reduced cancer cell proliferation through inhibition of c-Myc and Wnt signalling have been outlined above, once again highlighting POPDC1 as an interesting novel target for anti-cancer drug development. Its interactions with PP2A of the c-Myc pathway and potential intracellular regulation of LRP6 to reduce Wnt signalling (described above) may be of particular interest here.

### 3 | ROLE OF POPDC1 IN CELL ADHESION AND METASTASIS

Another important hallmark of cancer is the loss of cell adhesion, which allows cells to gain motility and spread to distant sites in the body (metastasise). Cancer cell metastasis presents one of the greatest hurdles in successful anti-cancer treatment, especially as secondary tumours preferentially arise in tissues that are very difficult to target with traditional therapy (e.g., brain and bone). It is estimated that metastasis is the cause of around 90% of cancer fatalities (Guan, 2015). This highlights why early intervention and prevention of metastasis is key to therapeutic success.

Metastasis occurs in four main stages: loss of cell adhesion; cytoskeletal remodelling and gain of cell motility through EMT; intravasation and entry into the vascular or lymphatic system; and finally, extravasation (re-entry into surrounding tissue at a distal site). POPDC1 proteins have been shown to play an important role in cell adhesion and cancer cell metastasis. The following will review the various signalling mechanisms and downstream targets of POPDC1 implicated in these processes.

#### 3.1 | Cell adhesion

As previously discussed, POPDC1 has been shown to play an important role in maintaining epithelial TJs and cellular adhesion by interacting with occludin, ZO-1, and the Wnt signalling pathway. The latter two prevent activation of ZONAB and β-catenin-induced gene transcription, respectively, which lead to cytoskeletal rearrangement, disruption of TJs, and increased cell motility (Parang et al., 2018; Russ et al., 2011). A loss of cell adhesion and TJ formation in the absence of POPDC1 expression has been demonstrated in experiments using POPDC1 knockouts (Parang et al., 2018) and mutated (K272/273 mutation preventing dimerisation) POPDC1 proteins (Kawaguchi et al., 2008). These highlight that the reduced expression of POPDC1 associated with cancer cells may be an important step in mediating loss of cell adhesion, the first stage in the metastatic cascade.

#### 3.2 | Epithelial-to-mesenchymal transition

A further key step in the metastatic cascade, the gain of cell motility, is a result of EMT, which has been shown to be key to progression in all cancer types (Dongre & Weinberg, 2019). Three types of EMT exist: types 1 and 2, respectively, are important for embryonic development and wound healing, while type 3 EMT has been shown to be a central component in cancer metastasis. The reverse process of EMT, mesenchymal-to-epithelial transition (MET), is undergone after extravasation into the tissue at a secondary site. In undergoing MET, cancer cells can regain adhesive properties and start proliferating to form secondary malignant tumour masses (Hanahan & Weinberg, 2011).
EMT is initiated by the expression of EMT-inducing transcription factors (EMT-TFs), for example, SNAIL, SLUG, and TWIST. These suppress the expression of proteins associated with the epithelial phenotype, such as E-cadherin, occludins, and α6β4 integrins, and activate expression of mesenchymal proteins such as N-cadherin, vimentin, fibronectin, and β1 and β3 integrins. Key activators of EMT-TFs in cancer cells are TGF-β, β-catenin via the Wnt signalling pathway, and the tumour micro-environment (D’Angelo et al., 2020; Dave et al., 2012). Examples of cytokines and chemokines released from the cancer-associated fibroblasts and immune cells of the tumour micro-environment include TGF-β, IL-6, EGF, VEGF, TNF-α, and hepatocyte growth factor (HGF) (Dongre & Weinberg, 2019).

Some carcinoma cells do not undergo complete transition to the mesenchymal state (unlike during embryogenesis [type 1 EMT] or wound healing [type 2 EMT]) but instead only undergo partial EMT (Aiello et al., 2018) to reach a quasi-mesenchymal state (Dongre & Weinberg, 2019). Instead of epithelial protein expression being completely suppressed, these cells express proteins associated with both the epithelial and mesenchymal state. The resulting quasi-mesenchymal phenotype seems to confer greater cell motility to allow metastasis than the fully mesenchymal phenotype (Aiello et al., 2018), and of course, these cells also display sufficient plasticity to return to an epithelial state when the secondary tissue is penetrated. Formation of quasi-mesenchymal cells also leads to further difficulties associated with EMT and cancer treatment, as after transformation into this state, cells become resistant to both traditional chemotherapy and immunotherapy. Chemotherapy resistance to oxaliplatin- and cisplatin-based drugs has been shown in breast, ovarian, colon, and PACs, as a result of suppression of apoptosis by EMT-TFs, as well as through CSC formation (see later) (Lim et al., 2013). A key point here is that CSCs arise somewhere along the EMT pathway, although the exact molecular mechanisms remain to be elucidated (Dongre & Weinberg, 2019). The role of CSCs in the progression of the malignant phenotype is discussed later in this review.

Mechanisms involved in the early stages of EMT, such as the loss of cell adhesion, have been shown to be regulated by POPDC1. The dysregulation and loss of POPDC1 expression in cancer cells (e.g., CRC and HCC) have been shown to promote EMT in several studies (Han et al., 2014; Han et al., 2019; Williams et al., 2011). Studies by Han et al. highlighted a correlation between POPDC1 loss, enhanced cell motility and invasion, and a decrease in E-cadherin expression and an increase in SNAIL1, TWIST (EMT-TFs), and vimentin (a mesenchymal cell marker). Increased levels of vimentin and acquisition of a mesenchymal phenotype in POPDC1 knockout cells were also observed by Kawaguchi et al. (2008). This is in accordance with the expression pattern associated with EMT induction described by Dongre and Weinberg (2019). Restoration of POPDC1 expression in CRC cell lines re-introduced an epithelial-like morphology (Williams et al., 2011), further supporting the findings that POPDC1 seems to play a key role in EMT switching.

### 3.3 Other factors influencing metastasis

#### 3.3.1 VAMP3

VAMP3 is a vesicular transport protein (SNARE protein) that aids cell migration by inserting transferrin and β1 integrin into the cell membrane (Benesh et al., 2013; Hager et al., 2010; Han et al., 2019). POPDC1 proteins interact with VAMP3 via their CTD, which experiments have mapped to a region after aa118 (Hager et al., 2010). As previously discussed, high levels of β1-integrin proteins in the cell membrane are associated with the mesenchymal cell type (Dongre & Weinberg, 2019). The POPDC1/VAMP3 interaction creates equilibrium between β1-integrin integration and recycling in the cell membrane to maintain cell function. If POPDC1 expression is suppressed in cancer cells, this interaction is lost, and VAMP3-mediated recycling of β1 integrin is impaired. A disruption in this equilibrium leads to higher concentrations of β1 integrin in the cell membrane, which promotes migration. Preventing the loss of POPDC1 proteins in cancer cells to maintain its interaction with VAMP3 may potentially provide a means to reduce cancer cell migration.

#### 3.3.2 GEFT/Rac1/Cdc42

Furthermore, as described previously, POPDC1 has been shown to negatively regulate GEFT (Smith et al., 2008), which in turn leads to reduced activation of Rac1 and Cdc42. These are members of the Rhofamily of GTPases and have been shown to induce membrane ruffling and lamellipodia, as well as filopodia and formation of actin microspikes (Guo et al., 2003). These processes increase cell motility and promote cancer metastasis. Moreover, Rac1 and Cdc42 increase the transcriptional activities of serum response elements (SREs) (e.g., Elk1 and SAP1) of the c-fos proto-oncogene, which promote proliferation and induce EMT. As increased GEFT activity has been shown to induce lamellipodia, filopodia, and actin cytoskeleton rearrangement through Rac1 and Cdc42 activity (Guo et al., 2003), preventing this activity by inhibiting the POPDC1/GEFT interaction may be beneficial to reduce cancer cell metastasis.

Despite Guo et al. (2003) showing that GEFT preferentially activates Rac1 and Cdc42 over RhoA, RhoA can be activated via other mechanisms, such as TJ downstream signalling pathways and the TJ-associated GEFH (Russ et al., 2011). RhoA activates ROCK, which in turn stimulates myosin light chain kinase (MLCK), causing cytoskeletal rearrangement and allowing cell migration (Williams et al., 2011). GEFH is usually sequestered to the TJ (via POPDC1 interaction) where it cannot activate RhoA (Han et al., 2015; Parang et al., 2018). Down-regulation of POPDC1 and loss of TJ formation in cancer cells results in increased RhoA activation through the mechanisms outlined above, thereby triggering migration.

Studies by Wang et al. (2014) have identified that miRNA-122 is under-expressed in HCC cells. Besides leading to down-regulation of POPDC1 (discussed previously), their studies also showed miRNA-122 to increase RhoA signalling and subsequently induce EMT.
Restoring miRNA-122 expression attenuated RhoA signalling, promoted MET, and led to reduced cellular migration (Wang et al., 2014). Hence, inhibiting the loss of miRNA-122 expression in cancer cells could therefore prevent metastasis not only by stabilising POPDC1 and maintaining the integrity of TJs but also by dampening down RhoA-induced EMT. The activation of the non-canonical Wnt signalling pathway has also been shown to activate both Rac1 and RhoA as a result of ROR-Fzd receptor complex activation (see Section 2.4). This switches on RhoA and Rac1 signalling, which activates the ROCK signalling cascade, whilst the latter activation of the G<sub>c</sub>-coupled Wnt5a receptor triggers an increase in intracellular Ca<sup>2+</sup> concentration (Zhan et al., 2017). All of the above trigger changes in actin polymerisation and cytoskeletal rearrangement, facilitating cell motility and metastasis.

In summary, combined activation of Wnt signalling, RhoA, Rac1, and Cdc42 promotes EMT, migration, invasion, angiogenesis, and cell proliferation (discussed previously). Hence, inhibiting GEFT, Cdc42, Rac1, RhoA, and Wnt signal activation is a promising approach in the treatment of cancer and will be outlined in the outlook below.

### 3.3.3 NDRG4

Studies by Benesh et al. (2013) have shown that n-Myc downstream-regulated gene 4 (NDRG4) binds to the CTD of POPDC1 (outside the Popeye domain in the region of aa300–aa357). This binding site is unique to the POPDC1 protein and is not found in the other cAMP binding proteins. NDRG4 is a known tumour suppressor (Amunjela et al., 2019), which represses cell proliferation, angiogenesis, metastasis, process extension, and invasion, and, like POPDC1, has been shown to also be down-regulated due to hypermethylation in cancer cells (Benesh et al., 2013). The interaction between POPDC1 and NDRG4 has previously been identified in epicardial cells. A loss of this interaction resulted in impaired fibronectin trafficking, prevention of VAMP3 docking to the cell membrane, and uncoordinated, non-directional cellular migration (Benesh et al., 2013). While evidence for this interaction in cancer cells has yet to be established, stabilising the POPDC1 protein to maintain POPDC1–NDRG4 interaction could be a useful approach to prevent the spread of cancer cells.

### 3.3.4 Netrin-1

Netrin-1 is considered an oncosgene, which inhibits apoptosis and promotes proliferation, differentiation, EMT, and metastasis (Han et al., 2015). Netrin-1 binds to four different receptors: deleted in CRC (DCC) and UNC5A, UNC5B, and UNC5C. Netrin-1 is up-regulated in many cancer types, especially when the tumours are high grade or metastatic, such as BC, LC, CRC, PAC, GC, and glioblastoma (Kefeli et al., 2017). Of the four netrin-1 receptors, DCC and UNC5B have been shown to function as tumour suppressors in the absence of their ligand (Kefeli et al., 2017).

The absence of netrin-1 allows DCC-mediated apoptosis through caspase activation, and the UNC5B receptor contains a binding site for p53 and enables p53-induced apoptosis. However, along with p53, the DCC and UNC5B receptors have been shown to be down-regulated in CRC and GC through gene methylation (Kefeli et al., 2017). Despite metastasis allowing the receptors to increase their distance from the netrin-1 ligand, their down-regulation stops them from inducing apoptosis to prevent this migratory step (Arakawa, 2004).

Netrin-1 also promotes other features of cancer, for example, angiogenesis, by exerting a similar effect on the endothelium as VEGF (Kefeli et al., 2017). It also influences proliferation and invasion, which have been shown to be mediated through DCC binding (Arakawa, 2004) and activation of the RhoA, Rac1, Cdc42, and PI3K signalling pathways (Ylivinkka et al., 2016). Clearly, while netrin-1 promotes activation of these pathways, POPDC1 mediates inhibition. Therefore, netrin-1 up-regulation and corresponding POPDC1 down-regulation are associated with a progressively more malignant phenotype, with both linked to downstream increases in RhoA, Rac1, and Cdc42 signalling in cancer cells.

Further evidence for netrin-1 promoting metastasis, invasion, and angiogenesis via PI3K/Akt and ERK signalling was demonstrated by Zhang et al. (2018) in NSLCC, whilst Han et al. (2015) showed this to occur in HCC and correlate with POPDC1 down-regulation. Subsequent re-expression of POPDC1 reduced the metastatic potential. Netrin-1 also up-regulates EGF expression. The EGFR signalling pathway has been separately shown to decrease POPDC1 expression (Amunjela & Tucker, 2017a). It is therefore a reasonable assumption that restoring POPDC1 expression and/or preventing its down-regulation by netrin-1 could provide a means of preventing cancer metastasis.

### 3.4 Outlook—Maintaining cell adhesion and inhibiting metastasis by stabilising POPDC1

From the data outlined above, limiting POPDC1 loss could prevent disassembly of TJs and loss of cell adhesion, metastasis, and cell spreading, as well as decreasing the EMT induced by POPDC1 loss. This could be achieved in three different ways: stopping POPDC1 down-regulation through miRNA-122 loss or increased netrin-1 signalling, increasing POPDC1 expression, or directly stabilising existing POPDC1 proteins. The latter may, for example, reduce changes in cell migration if its interaction with NDRG4 and VAMP3 to promote recycling of β1 integrin were maintained.

The important role played by the RhoGTPase family in cancer has been discussed in detail above and highlights that this protein family presents itself as an ideal candidate for targeted cancer therapy. As the globular structure of the RhoGTPases has been deemed “undruggable,” alternative approaches need to be investigated (del Mar Maldonado & Dharmawardhane, 2018). Various low MW inhibitors that target different parts of the RhoGTPase signalling pathway have already been developed or are currently undergoing clinical trials (Lin & Zheng, 2015). The most promising compounds act by disrupting the Rho-GEF interaction to inhibit RhoGTPase activation. These approaches enhance the tumour response to classic anti-cancer
agents such as cisplatin (e.g., in lung, renal, and oesophageal squamous carcinoma) or EGFR/HER2 therapy in BC (Clayton & Ridley, 2020; del Mar Maldonado & Dharmawardhane, 2018; Zhang et al., 2020). Moreover, Rho-GEF inhibitors have been shown to reduce progression of some cancers, including BC, leukaemia, melanoma, and fibrosarcoma after EHop-016 treatment, and various specific inhibitors are already available for treatment (e.g., NSC23766, TBOPP, and EHop-016 [Rac1 specific], CASIN [Cdc42 specific], and LARG [RhoA specific]) (Clayton & Ridley, 2020; Lin & Zheng, 2015; Tajiri et al., 2017).

As Rho-GEF interaction inhibitors have shown promise in cancer drug development (del Mar Maldonado & Dharmawardhane, 2018), stabilising the POPDC1 protein to maintain its negative regulation of GEFT and inhibition of RhoGTPase activation may be a novel approach in the development of new anti-cancer treatments. While RhoGTPase proteins can be activated by numerous GEFs, not solely GEFT, inhibiting this one interaction may at least be a start in reducing Rac1, Cdc42, and RhoA signalling in cancer cells. When combined with the other corrections this stabilisation may achieve across other malignant cascades, this could prove a highly effective approach. Limited success has been demonstrated when the Rho/GEFT pathway is targeted by preventing Rho-effector protein interaction. Therefore, targeting POPDC1 stabilisation may present itself as a viable option for reducing Rho/GEFT activation and enhancing POPDC1 presence, thereby maintaining its tumour-suppressive activity.

To summarise, stabilising the POPDC1 protein in cancer cells could prevent metastasis, one of the main causes of cancer-associated deaths. First, however, more evidence for an interaction between PODPC1 and these proteins in cancer cells is required before these approaches can be further explored.

### 4 | POPDC1 AND CSCs—RELATIONSHIP BY ASSOCIATION

A malignant neoplasm is a heterogeneous tissue consisting of many different cell types, including cancer-associated fibroblasts, cancer cells, epithelial cells, pericytes, and inflammatory immune cells (Hanahan & Weinberg, 2011), which are involved in different aspects driving tumour progression. One of these cell types can self-renew and differentiate into all types of cancer cells (pluripotency) (Du et al., 2019). These cells, known as CSCs, are implicated in the main difficulties of therapeutic cancer treatment. The formation of CSCs and quasi-mesenchymal cells somewhere along the EMT axis has already been discussed in detail, and this process is key to understanding anti-cancer drug resistance. CSCs are resistant to traditional cytotoxic chemotherapy agents due to their high expression of drug extrusion pumps, such as P-glycoprotein (Shibue & Weinberg, 2017; Zhang et al., 2017) and, similar to quasi-mesenchymal cells, possess mechanisms to avoid immune system detection. Furthermore, CSCs can also gain motility and undergo the process of metastasis outlined above and lie quiescent at local or distal sites for many years, before eventually re-entering the cell cycle leading to the development of a secondary tumour. It is important to note that some questions have been raised recently about the existence of CSCs in solid tumours, where, instead, tumour-initiating cells (TICs) have been suggested as a preferred term. This debate is beyond the scope of this article, and the reader is referred to other reviews (Machida, 2017; Magee et al., 2012; Suraneni & Badeaux, 2013) for further discussion of this point. Anti-cancer drugs that specifically target CSCs could potentially solve these key issues driving disease progression (Zhang et al., 2017), which perhaps makes CSCs the most promising target of all for developing new anti-cancer treatments (Yang et al., 2020).

#### 4.1 | Signalling proteins in CSCs

CSCs use the same signalling pathways as normal stem cells (Yang et al., 2020), including the hedgehog, NF-κB, JAK–STAT and PI3K/Akt/mTOR pathways, and the Wnt pathway (Yang et al., 2020). The latter is a critical signalling pathway in CSCs, and aberrant Wnt signalling has been demonstrated in various leukaemias, BC, CRC, lung cancer, PC, and ovarian cancer (Zhong & Virshup, 2020). A first generation of extracellular inhibitors of Wnt signalling have already entered clinical trials, for example, vantictumab, a monoclonal antibody that inhibits the Frizzled receptor (Du et al., 2019).

The Wnt signalling pathway regulates multiple pathways that give CSCs their unique properties. For one, it ensures pluripotency and regulates CSCs differentiation. Furthermore, Zhan et al. (2017) also demonstrated that the Wnt pathway increased β-catenin-mediated expression of the TERT gene. TERT, telomere reverse transcriptase, is an enzyme that prevents telomere shortening in CSCs, giving them replicative immortality, another important hallmark of malignancies (Hanahan & Weinberg, 2011). Signalling molecules of the tumour environment have been shown to maintain CSC properties via activation of the canonical and non-canonical Wnt pathways in CRC and BC (Yu et al., 2013; Zhan et al., 2017). Furthermore, in APC-deficient cancers (e.g., colon adenoma or CRC), the Wnt signalling pathway is constitutively active, because loss of the APC gene leads to an increase in GEF-mediated activation of Rac1, which in turn stimulates NF-κB (Myant et al., 2013). This co-activation, which increases Wnt signalling, not only promotes cell proliferation but has also been shown to initiate de-differentiation of normal intestinal cells into CSCs (Schwitalla et al., 2013).

The interaction of the POPDC1 protein with GEFT, an activator of Rac1, has been extensively discussed above. If the interaction between POPDC1 and GEFT could be maintained in CSC, it could reduce the activation of Rac1 and Wnt signalling outlined in this section. Despite the possibility of Rac1 being activated by other GEFs, reducing its activation by GEFT could provide a means to at least dampen down Wnt signalling in CSCs. It has also been highlighted in this review that POPDC1 negatively regulates Wnt signalling through its interaction with the LRP6 receptor, suggesting that POPDC1 stabilisation may have dual benefit. In summary, POPDC1 stabilisation introduces a new therapeutic approach to inhibiting the Wnt pathway intracellularly, at a point of integration between multiple signalling pathways.
4.2 Transcriptional regulation in CSCs

The most important transcription factors in CSCs include Sox2, Nanog, Oct4, KLF4, and Myc (Yang et al., 2020), of which the latter is involved in regulating cell metabolism, self-renewal, and proliferation. Its expression is often dysregulated in CSCs (Chen et al., 2018), with high levels of c-Myc being achieved by reducing the amount of c-Myc ubiquitination and subsequent degradation, as well as increased Wnt signalling. c-Myc also promotes the hallmarks listed above and the formation of invasive CSCs.

As previously discussed, POPDC1 interacts with the regulatory PR61α subunit of PP2A and increases the amount of c-Myc targeted for proteasomal degradation, as well as the Wnt signalling pathway to decrease β-catenin activation. Various experimental data suggest that this PP2A–PR61α interaction with the Wnt and c-Myc pathways regulates renewal and proliferation of stem cells (Thompson & Williams, 2018), yet a direct link to POPDC1 involvement remains to be established. However, if a link was confirmed, by association, one could hypothesise that POPDC1 may be key in regulating c-Myc expression by two different pathways (increasing PP2A activity and reducing Wnt signalling) in CSCs. Stabilising the POPDC1 protein to maintain this action may be able to reduce CSC proliferation by two separate mechanisms, making it a highly promising therapeutic target.

4.3 EMT and differentiation

Two important steps in the metastatic cascade have been discussed in this review: the loss of cell adhesion, which induces EMT, and the emergence of CSCs along the EMT axis, contributing to anti-cancer drug resistance and the formation of secondary tumours previously mentioned (Dongre & Weinberg, 2019).

POPDC1 down-regulation has been shown to result in loss of cell adhesion and EMT (Williams et al., 2011), and EMT-mediated induction of stemness has been evidenced in mammary carcinoma cells (Mani et al., 2008) and intestinal stem cells (ISCs) (Reddy et al., 2016). Although a link has yet to be established, the loss of POPDC1-inducing TJ disassembly and EMT could be driving the generation of CSCs. Thus, POPDC1 membrane stabilisation in cancer cells could potentially prevent EMT-induced formation of CSCs. Studies by Reddy et al. (2016) on ISCs showed that POPDC1 is down-regulated after radiotherapy treatment. This correlated with an increase in Wnt signalling, evidenced by an increase in Wnt ligand concentration, Frizzled receptor activation, and downstream target gene transcription, which are key to ISC proliferation. Subsequent rapid expansion of the ISC population was also observed, leading to the conclusion that POPDC1 down-regulation and ensuing stem cell proliferation, in response to radiation therapy may be an underlying cause of resistance to radiotherapy treatment. The same phenomenon has also been observed as a result of POPDC3 down-regulation in other cancers such as HNSCC, lung cancer, and oesophageal cancer (Table 1) (He et al., 2019). Stabilising these two isoforms of POPDC1 in the respective cancers may therefore reduce CSC formation and resistance to radiotherapy.

In addition, NF-κB-induced Wnt signalling in intestinal epithelial cells has also shown to promote dedifferentiation of normal epithelial cells into tumour-initiating stem cells (Schwitalla et al., 2013). One could hypothesise that the POPDC1 protein could be involved in this Rac1/NF-κB co-activation of the Wnt signalling pathway as described above and that preventing POPDC1 loss in intestinal epithelia could result in reduced cell dedifferentiation into tumour-initiating CSCs.

The presence of POPDC1 proteins in CSCs and possible interaction with CSC signalling remains a relationship by association and still needs to be demonstrated experimentally. However, evidence shows that POPDC1 proteins play a crucial role in regulating c-Myc and Wnt signalling pathways in normal cancer cells, and it is also known that CSCs use exactly the same signalling mechanisms (Dongre & Weinberg, 2019; Yang et al., 2020). If POPDC1 protein expression is also down-regulated in CSCs, then stabilising the POPDC1 protein could be a useful therapeutic approach to directly inhibit cell proliferation and metastasis in CSCs.

5 CONCLUDING REMARKS

The many ways in which PODPC1 acts as a tumour suppressor in cancer cells by mediating cell adhesion and suppressing proliferation and metastasis through numerous signalling pathways have been discussed (Figure 2). However, its tumour-suppressive actions are often lost due to down-regulation in various types of cancer, including HCC, CRC, PC, BC, NSCLC, HNSCC, GC, and glioma (Table 3).

Many downstream targets of POPDC1, such as ZO-1, GEFH/GEFH, Wnt signalling, c-Myc, VAMP3, and netrin-1, are already established targets for drug development. However, their ubiquitous expression may produce wide-ranging side effects. Hence, specifically stabilising POPDC1 in cancer cells to maintain interaction with these effectors may present itself as a novel, targeted approach to developing anti-cancer drugs. In line with the data presented above, POPDC1 stabilisation could have a broad effect by making subtle changes to many different signalling pathways to promote cell adhesion, as well as reduce cancer cell proliferation and metastasis.

Future studies should aim to develop a low MW compound that could stabilise POPDC1 and determine whether this would have the desired anti-tumour effects suggested above. Complete mapping of the interaction between POPDC1 and its target proteins to specific amino acid sequences will also be a vital step towards therapeutically targeting all POPDC1 downstream effectors. Further exploration of how POPDC1/PR61α interaction may have a dual effect on both Wnt and c-Myc signalling may also be promising. Finally, investigation of whether POPDC1 is expressed in CSCs is crucial for confirming the possibilities of CSC targeting outlined in this review. The potential roles that POPDC1 may play in other hallmarks of cancer, such as angiogenesis, have also been touched upon and highlight the importance of this protein across a broad spectrum of characteristics of this disease. Further study may therefore unveil additional roles of POPDC1 in the progression of the malignant phenotype.
5.1 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos, et al., 2019; Alexander, Fabbro, et al., 2019a, b; Alexander, Kelly, et al., 2019; Alexander, Mathie, et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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