ID4 controls luminal lineage commitment in normal mammary epithelium and inhibits *BRCA1* function in basal-like breast cancer

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Abstract

Inhibitor of differentiation (ID) proteins are key regulators of development and tumorigenesis. One member of this family, ID4, controls lineage commitment during mammary gland development by acting upstream of key developmental pathways. Recent evidence suggests an emerging role for *ID4* as a lineage-dependent proto-oncogene that is overexpressed and amplified in a subset of basal-like breast cancers (BLBCs), conferring poor prognosis. Several lines of evidence suggest ID4 may suppress *BRCA1* function in BLBC and in doing so, define a subset of BLBC patients who may respond to therapies traditionally used in *BRCA1*-mutant cancers. This review highlights recent advances in our understanding of the requirement for ID4 in mammary lineage commitment and the role for ID4 in BLBC. We address current shortfalls in this field and identify important areas of future research.

Key Words

- ▶ basal-like breast cancer
- ▶ ID4
- ▶ inhibitor of differentiation 4
- ▶ BRCA1
- ▶ lineage dependent oncogene

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Introduction

Inhibitor of differentiation (ID) proteins are a family of four (ID1–4) helix-loop-helix (HLH) transcriptional regulators. They demonstrate distinct tissue-specific expression patterns and regulate various cell processes including transcription, differentiation and cell cycle progression. Recent reviews have eloquently addressed the biological requirement for ID proteins (Lasorella *et al.* 2014, Nair *et al.* 2014). In recent years, we have gained insight into the role of one member of this family, ID4, in mammary gland development and tumorigenesis.

ID4 controls mammary epithelial cell lineages

The mammary gland as a model of epithelial stem cell biology

Mammary gland development is almost exclusively postnatal (reviewed in Watson and Khaled 2008). As such, it is a particularly unique model for studying epithelial stem cell biology and differentiation. Murine models are often used to study the mammary gland as these developmental processes are highly conserved with humans. The pubertal mammary

gland is characterised by a branching ductal structure culminating in terminal end buds (TEBs). The cap cells residing at the ends of these TEBs are characterised by high proliferation and stem/progenitor activity (Williams & Daniel 1983, Bai & Rohrschneider 2010). These cells drive invasive ductal branching through the mammary fat pad during puberty.

The mammary gland is continually remodelled as a result of external hormonal (de Candia et al. 2006) and growth factor signals (Bouras et al. 2008, Li et al. 2008). These cues result in expansion of the stem/progenitor cell population during puberty and differentiation during pregnancy and lactation. The resulting bilayered ducts are comprised predominantly of two terminally differentiated cell types, the inner luminal cell layer and the surrounding basal/myoepithelial cell layer, which are enriched for mammary stem cell activity (MaSC) (Williams & Daniel 1983, Best et al. 2014, Junankar et al. 2015). Cells of this basal layer will henceforth be referred to as basal cells.

Terminally differentiated mammary cell lineages can be characterised by distinct molecular signatures (Lim et al. 2009), coordinated by the activity of several transcription factors. A number of transcriptional regulators required for terminal differentiation of the mammary gland are breast cancer type-1 susceptibility protein (BRCA1), E74-like factor 5 (ELF5), GATA-binding protein 3 (GATA3) and Forkhead box protein M1 (FOXM1). Combined with alterations to Notch signalling, these factors affect luminal progenitor differentiation and terminal differentiation of ductal/alveolar luminal cells (Kouros-Mehr et al. 2006, Liu et al. 2008, Oakes et al. 2008, Carr et al. 2012). In contrast, the transcriptional mediators of basal cell differentiation are relatively poorly understood. This is partially a result of difficulties in the purification and molecular and biochemical characterisation of these cells (Lindvall et al. 2006, Zeng & Nusse 2010).

ID4 in the mammary gland

Increasing evidence supports a central role for ID4 in regulating mammary cell proliferation and lineage commitment. Lim and coworkers (Lim et al. 2009, Lim et al. 2010) purified and analysed the transcriptional signatures of the main cell populations comprising the mammary gland: luminal progenitors (LPs), committed/mature luminal (ML) and basal cells. Comparison of the basal population with either the LP or ML cells revealed differential expression of many genes. Amongst them,

ID4 was one of the highest differentially expressed genes specific to basal cells of both human and murine origin (Lim et al. 2009, 2010).

Research by Junankar and coworkers and Best and coworkers (Junankar et al. 2015, Best et al. 2014) has subsequently shown almost exclusive localisation of ID4 within the basal cell compartment, particularly within the cap cells of the TEBs (Williams & Daniel 1983). Interestingly, a small proportion of luminal progenitor cells have also been reported to express ID4 during pubertal development (Best et al. 2014). ID4 controls luminal commitment and mammary stem/progenitor cell self-renewal by acting as a negative regulator of several key pathways involved in luminal fate specification, namely Notch signalling, *BRCA1*, *ELF5*, *ERα* and *FOXA1* (Best et al. 2014, Junankar et al. 2015). The ID4-mediated inhibition of *ELF5* occurs indirectly via Notch signalling (Junankar et al. 2015), while ID4 has been shown to inhibit *ERα*, *FOXA1* and *BRCA1* expression by direct interaction with the promoters of these genes (Beger et al. 2001, Best et al. 2014). As ID4 does not have a basic DNA-binding domain (reviewed in Sharma et al. 2015), it is likely that it regulates gene expression by forming a complex with other transcriptional regulators. The identity of these transcriptional regulators is currently unknown. Loss-of-function studies have confirmed the requirement for ID4 in ductal morphogenesis in the developing pubertal mammary gland (Dong et al. 2011, Best et al. 2014, Junankar et al. 2015), with ID4-positive cells repopulating the adult mammary gland at a greater frequency than ID4-negative cells demonstrating the intrinsic stem cell activity of the ID4-positive basal cells (Junankar et al. 2015). ID4-knockout mice also have delayed ductal morphogenesis further signifying the requirement for this transcription factor in mammary gland development (Dong et al. 2011, Junankar et al. 2015).

ID4 interacts with important developmental regulators ERα and BRCA1

ID4, ERα and BRCA1 in the mammary gland

Transcriptional regulation of mammary gland development has been broadly studied. ERα, for example, is a key controller of cell proliferation during mammary gland development (Korach et al. 1995, Feng et al. 2007). Depletion of ERα or disruption of its signalling network results in the formation of rudimentary mammary gland structures (Korach et al. 1995, Mallepell et al. 2006).

Similarly, ID4 is expressed in hormonally regulated tissues including the breast, ovaries and prostate and is required for normal development (Shan *et al.* 2003, Dong *et al.* 2011, Sharma *et al.* 2013, Best *et al.* 2014, Junankar *et al.* 2015). ID4 is stimulated by progesterone receptor (PR) activity through direct binding of ligand-activated PR to the ID4 promoter (Fernandez-Valdivia *et al.* 2008). Mammary ID4 expression fluctuates through the oestrous cycle (S Junankar and A Swarbrick, unpublished observations) and murine ID4-knockout models show impaired production of oestradiol synthesis enzymes (Best *et al.* 2014). Other studies identify β -oestradiol as an inhibitor of ID4 expression (Beger *et al.* 2001), suggesting a complex regulatory interaction role between ID4 and ER α that remains to be fully elucidated.

Conversely, Best and coworkers suggest that ID4 inhibits the expression of both ER α and its cofactor FOXA1 in the developing mammary gland (Best *et al.* 2014). This occurs through interaction of ID4 with the promoters of these genes. When ID4 is depleted, ER α expression is reactivated in the myoepithelial and luminal progenitor compartments, cell populations that do not express ER α under normal conditions (Best *et al.* 2014). This correlates with data showing almost exclusive localisation of ID4 expression to the ER α -negative basal cell compartment (de Candia *et al.* 2006, Junankar *et al.* 2015). These findings have led many researchers to hypothesise that ER α signalling inhibits ID4 in the developing mammary gland and loss of ID4 may be an early event in the formation of ID4-negative ER α -positive breast cancer (Beger *et al.* 2001, de Candia *et al.* 2006, Junankar *et al.* 2015).

BRCA1, a regulator of mammary stem cell fate, is required for the differentiation of basal cells into luminal cells (Liu *et al.* 2008). BRCA1 depletion results in blocked epithelial differentiation (Liu *et al.* 2008). Negative regulation of BRCA1 by ID4 is one of the mechanisms through which ID4 maintains the basal population (Junankar *et al.* 2015).

ID4, ER α and BRCA1 in breast cancer

ID4, ER α and BRCA1 have been studied for their roles in normal breast development and cancer. *BRCA1*, the breast and ovarian cancer susceptibility gene, is responsible for the majority of hereditary breast cancer cases ((Lakhani *et al.* 2005, Turner *et al.* 2007) and reviewed in Mavaddat *et al.* 2010). BRCA1 and ER α mRNA expression have been shown to correlate in sporadic breast cancers (Roldán *et al.* 2006), while ID4 is negatively correlated to both

BRCA1 and ER α (Roldán *et al.* 2006, Thike *et al.* 2015). This phenomenon has been suggested to occur through ER α inhibition of ID4 in a luminal breast cancer cell line (Beger *et al.* 2001). However, the relevance is unclear, as in clinical breast cancer, ID4 expression is exclusive to ER α -negative subtypes of breast cancer (Crippa *et al.* 2014). This evidence has led researchers to suggest that ID4 loss may be important in the development of ER α -dependent breast cancers (de Candia *et al.* 2006), and conversely, ID4 may suppress BRCA1 and ER α in ID4-positive BLBC (Best *et al.* 2014), resulting in poor survival outcomes (Junankar *et al.* 2015, Thike *et al.* 2015).

Furthermore, ID4 depletion in a basal-like MMTV-Wnt-1 mammary tumour model results in re-expression of ER α and activation of the ER α signalling network, indicated by the expression of FOXA1 (Best *et al.* 2014). This data suggest that by inhibiting ID4 in BLBC, we may be able to reactivate luminal pathways, potentially rendering them susceptible to hormonal therapies such as ER α inhibition. Testing in more BLBC models is required to confirm these findings and to determine their clinical ramifications.

Cell of origin

Our understanding of the normal cells from which cancer originates is incomplete. In recent years, we have begun to identify the molecular and cellular characteristics that must be acquired to enable malignant transformation. Gain of proliferative and angiogenic capacity, resistance to tumour suppression and cell death as well as acquired invasive and metastatic potential are well-established hallmarks of cancer (Hanahan & Weinberg 2011). However, it is unclear which cells are primed to acquire these advantages and whether the cell of origin predisposes patients to particular disease aetiologies.

Increasing evidence suggests that cancer phenotype is in part dictated by the cell of origin ((Barker *et al.* 2009) and reviewed in Visvader 2009). Lineage tracing experiments have demonstrated the role of unipotent and multi-potent stem cells in maintaining epithelial tissue through the generation of terminally differentiated cell types (Shackleton *et al.* 2006, Stingl *et al.* 2006, Barker *et al.* 2009, Van Keymeulen *et al.* 2011, Rios *et al.* 2014, Wang *et al.* 2015). By identifying the cell of origin, we will be able to examine, with greater precision, the molecular and biological changes enabling cancer initiation and progression and identify targeted approaches to treat cancers in early development.

Cell of origin in BLBC

Breast cancer is a heterogeneous disease that can be stratified into at least five major subtypes: luminal A, luminal B, *Her2*-enriched, basal-like and normal-like (Perou et al. 2000). Each has unique molecular and genomic features and histopathology that effect different clinical outcomes (Prat & Perou 2011). Basal-like breast cancer (BLBC) is a poorly characterised, heterogeneous disease that accounts for ~18% of all breast cancer diagnoses (Sørli et al. 2001, Prat & Perou 2011, Cancer Genome Atlas Network 2012). Patients are diagnosed at an earlier age than other subtypes, with aggressive, high-grade tumours and often relapse with chemotherapy resistance.

The BLBC subtype is enriched for patients harbouring mutations in tumour suppressor protein 53 (*TP53*) and *BRCA1* ((Lakhani et al. 2005, Turner et al. 2007, Bertheau et al. 2013) and reviewed in Mavaddat et al. 2010). This subtype has been defined using molecular analysis of patient samples and represents a subset of the triple-negative subtype. This has been extensively reviewed in recent years (Badve et al. 2011, Prat et al. 2013). BLBCs generally lack expression of ER α , PR and human epidermal growth factor receptor 2 (*Her2*) and are marked by the presence of cytokeratin 5/6 and/or epidermal growth factor receptor (EGFR). Patients are unsuitable for currently available targeted therapeutics (Perou et al. 2000, Sørli et al. 2001). This contributes to the poor survival associated with this disease, which is compounded by considerable molecular and clinical heterogeneities. Approximately, 30–50% of patients relapse within 3–5 years, while the remaining patients have good long-term survival (reviewed in Rakha et al. 2008). Effective stratification of patients through predictive biomarker discovery is crucial to improving treatment options and patient survival, as is the discovery of new therapeutic vulnerabilities in BLBC.

Basal cytokeratins are highly expressed in BLBC ((Prat et al. 2013) and reviewed in Gusterson et al. 2005). Accordingly, initial research suggested that mammary stem (MaSC) or basal cells were the possible cells of origin for BLBCs. In support of this hypothesis, *BRCA1* depletion blocks epithelial differentiation, as mentioned above (Liu et al. 2008), and increases the population of genetically unstable stem/progenitor cells, a known predisposing event to the formation of BLBC ((Miki et al. 1994, Liu et al. 2008) and reviewed in Turner et al. 2007).

The origination of BLBC from the MaSC population is also supported by study of ID4 in the transformation process. ID4 is localised to the basal cell compartment in

the normal breast, and high ID4-expressing cells from a murine cell line possess upregulation of MaSC-associated genes (Junankar et al. 2015). Furthermore, human BLBCs with high ID4 expression closely resemble the MaSC molecular signature (Junankar et al. 2015), suggesting that ID4 may play an important role in progression from normal MaSCs to BLBC.

Conversely, recent studies have hypothesised LPs as the cell of origin for BLBCs (Lim et al. 2009, Molyneux et al. 2010). To uncover the cell of origin, Lim and coworkers (2009) used microarray analysis to investigate the gene expression signatures of sorted mammary populations: basal, luminal progenitor (LP) and mature luminal (ML) cells (Lim et al. 2009). These were compared with pre-neoplastic normal breast tissue from patients with *BRCA1* mutations and with the different breast cancer subtypes. High similarity was observed between the LP population and *BRCA1*-mutant pre-neoplastic tissue. Furthermore, when compared with different breast cancer subtypes, the LP population most closely resembled the BLBC subtype. Comparative analysis of *BRCA1* wild-type and mutant patients demonstrated an expansion of the LP population in the *BRCA1*-mutant cases (Lim et al. 2009). Taken together, these findings suggest that the LP is the precursor for *BRCA1*-mutant BLBC. Though Lim and coworkers do not provide direct evidence that BLBCs originate from the LP population, Molyneux and coworkers (2010) have subsequently directed *BRCA1* depletion to either predominantly the basal (consisting of basal stem cells) or luminal (consisting of LP cells) mammary epithelial cells, and showed that both conditions resulted in the formation of tumours that possessed molecular features resembling BLBC. However, only the tumours resulting from *BRCA1*-depleted luminal progenitor cells recapitulated the histological features of BLBC (Molyneux et al. 2010). *BRCA1* loss in basal cells instead generated malignant adenomyoepitheliomas, a rare breast cancer known to originate from stem cells (Molyneux et al. 2010).

Though the above evidence appears to support two opposing hypotheses, the weight of evidence suggests that BLBCs originate from the LP population, potentially from the small proportion of ID4-positive LP cells (Best et al. 2014), and a proportion of these tumours de-differentiate to acquire MaSC-like features (Fig. 1). This plasticity during transformation has been described in BLBC previously (Chaffer et al. 2013) and has been recently reviewed (Blanpain & Fuchs 2014).

As described above, ID4 expression is high in the basal population and low in the LP population (Fig. 1)

Figure 1

Cell of origin for ID4 $\pm$ BLBC. (1) ID4 controls lineage commitment in the developing mammary gland through regulation of key lineage commitment genes *Notch*, *ER*, *ELF5* and *BRCA1*. (2) Sophisticated murine models and clinical data suggest that transformation of normal mammary cells may occur through three pathways: (A) ID4 $^{+}$ common progenitor/stem cells may give rise to ID4 $^{+}$ basal-like breast cancers. This idea largely originates from molecular analysis of patient samples, where ID4 $^{+}$ BLBCs possess transcriptional signatures resembling mammary stem cells (Junankar et al. 2015). (B and C) ID4 $^{-}$ luminal progenitors may form

(Lim et al. 2009, Molyneux et al. 2010, Junankar et al. 2015). We propose that ID4-positive BLBCs originate from LP cells, and gain ID4 expression and gene amplification (Turner et al. 2004, Molyneux et al. 2010). This results in de-differentiation to an MaSC-like state resulting in similarities between the BLBC and MaSC transcriptomes (Junankar et al. 2015). The genetic alterations acquired during malignant transformation may account for the gene expression heterogeneity marking the ID4-positive BLBC compared with the ID4-negative BLBC (Junankar et al. 2015). This has implications for diversity in response to therapy and clinical outcome that is observed in BLBC.

ID4: a lineage-dependent oncogene in basal-like breast cancer

Development gone awry

Elucidating the role of key transcriptional regulators and lineage fate commitment genes has enabled incredible insight into tumorigenesis. Regulators of lineage commitment often become deregulated during transformation, driving tumour growth and progression. These factors are termed lineage-dependent oncogenes (reviewed in Garraway and Sellers 2006). As such, by exploiting these oncogene dependencies, we may uncover new therapeutic targets. Several transcription factors controlling luminal lineage commitment, such as GATA3 and BRCA1, are potent breast tumour suppressors that are aberrantly expressed or mutated in breast cancer (Kouros-Mehr et al. 2006, Asselin-Labat et al. 2007, Chen et al. 2010, Carr et al. 2012). Parallel to the developmental importance of ER α , as described above, researchers have shown increased levels of ER α in the benign breast

either ID4 $^{-}$ or ID4 $^{+}$ BLBC. Molecular comparison of luminal progenitors with breast cancer subtypes reveals close association with the BLBC subtype. However, analysis of this mixed basal-like subtype (including ID4 $^{-}$ and ID4 $^{+}$ tumours) may complicate the findings. We hypothesise that the luminal progenitor population gives rise to ID4 $^{-}$ and ID4 $^{+}$ BLBC, the ID4 $^{-}$ BLBC resembling the luminal progenitors and the ID4 $^{+}$ BLBC resembling a de-differentiated mammary stem cell signature. We are currently unable to distinguish the ID4 $^{+}$ BLBC derived from luminal progenitors with the transformation events occurring in (A). It has been shown that transformation seen in (A) and (C) results in more aggressive, poorly differentiated tumours (Junankar et al. 2015). In (C), ID4 is gained through unknown mechanisms. Evidence gathered from murine models identifies *BRCA1* mutation in luminal progenitor cells as the predisposing event to the formation of ID4 $^{+}$ BLBCs. Similarly, clinical data identify preferential ID4 amplification in *BRCA1*-mutant cases (Prat et al. 2014), suggesting an advantage to ID4 gain in tumorigenesis. Conversely, *BRCA1* mutation in common progenitor/stem cells results in the formation of ID4-positive adenomyoepitheliomas, a rare malignancy (Molyneux et al. 2010). ID4 $^{+}$, ID4-positive; ID4 $^{-}$, ID4-negative.

Table 1 Deciphering the relationship between ID4 and BRCA1 in breast cancer.

Study	Findings	Type of evidence	Limitations	Strengths
Beger et al. (2001)	Identified ID4 as a direct inhibitor of the <i>BRCA1</i> promoter, affecting <i>BRCA1</i> RNA expression β-oestradiol treatment inhibits ID4 and stimulates <i>BRCA1</i> RNA expression	Ovarian cancer cell line, luminal and <i>Her2</i> -enriched breast cancer cell lines Generated ribozyme library to identify promoter interaction RNA expression	The authors suggest that ID4 protein effects <i>BRCA1</i> gene expression; however, only ID4 RNA expression levels are analysed (RNA and protein expression do not always correlate (Gry et al. 2009))	Unbiased approach to identify regulators of <i>BRCA1</i> RNA expression
Welsh et al. (2002)	<i>ID4</i> gene expression is upregulated by inducible <i>BRCA1</i> expression construct in embryonic kidney cell line ID4 and <i>BRCA1</i> expression are positively correlated ID4 expression inversely correlated with <i>BRCA1</i>	Paraffin fixed tissue, immunohistochemistry Primary infiltrating ductal breast tumours and primary ovarian adenocarcinomas	Study was conducted in a luminal and <i>Her2</i> -Enriched breast cancer cell lines. We and others observe absence of ID4 expression in luminal breast cancer and an importance for ID4 in BLBC (De Candia et al. 2006 , Junankar et al. 2015)	Large cohort of 104 breast cancers
Roldán et al. (2006)		RNA expression from fresh tissue Sporadic breast cancer patients Majority of patients post-menopausal	Embryonic kidney cell line used for <i>BRCA1</i> overexpression (the authors note a <i>BRCA1</i> -mutant cell line was preferable but technically challenging)	
Turner et al. (2007)	<i>BRCA1</i> promoter is methylated at a similar rate in BLBC and non-BLBC <i>BRCA1</i> RNA expression was two-fold lower in BLBC suggesting other regulatory factors ID4 RNA expression was increased by 9.1-fold in BLBC ID4 promoter hypomethylation correlates with triple-negative breast cancer No association with <i>BRCA1/2</i> methylation ID4 amplification is significantly associated with <i>BRCA1</i> -mutant BLBC compared with <i>BRCA1</i> wild-type BLBC	Methylation of <i>BRCA1</i> promoter with methylation specific PCR (MSP) RNA expression Comparison of 20 BLBCs compared to non-BLBC controls CpG island methylation analysis	Non-subtype specified Would be important to measure protein expression levels (as above, RNA and protein do not always correlate (Gry et al. 2009)) Small cohort of 20 BLBC patients Would be important to measure protein expression levels (as above, RNA and protein do not always correlate (Gry et al. 2009))	
Branham et al. (2012)			Small sample size of 28 triple-negative patients Analysed approximately 2–3 probes	
Prat et al. (2014)		The Cancer Genome Atlas public microarray database (http://cancergenome.nih.gov/)	Small sample size (14 <i>BRCA1</i> -mutant BLBC and 74 <i>BRCA1</i> wild-type BLBC) Imprecise genomic techniques used to call amplification Did not examine <i>BRCA1</i> in BLBC Only examined <i>BRCA1</i> RNA expression	
Junankar et al. (2015)	ID4 controls <i>BRCA1</i> RNA expression through normal differentiation	Murine breast cell line with ID4 overexpression		

Review		L A Baker et al.	ID4 inhibits BRCA1 in basal breast cancer	23:9	R387
Crippa et al. (2014)	ID4 expression is inversely correlated with miR-342 and BRCA1	BRCA1-mutant BLBC and BRCA1-wild-type BLBC/Claudin-low cell lines	Only examined RNA expression in patient cohorts	40 familial cases	Large study of 699 patients
	Overexpression of miR-342 inhibits ID4 and increases BRCA1 RNA expression	Protein and RNA expression			
	ID4 acts as an upstream regulator of BRCA1 in ER-negative breast cancer cell lines				
Thike et al. (2015)	ID4 expression inversely correlated with BRCA1	Protein expression using tissue microarrays		183 primary breast cancers (71 triple negative breast cancers)	
Korlimarla et al. (2016)	The ratio of BRCA1 to ID4 expression is a better marker of triple negative breast cancers than either protein/gene alone	RNA and protein expression of BRCA1 and ID4 112 hormone receptor positive/Her2-negative, 71 triple-negative cases			

epithelium predisposes women to develop breast cancer (Khan et al. 1998). This gene controls the growth of luminal subtypes, accounting for ~70% of all breast cancers (reviewed in Lumachi et al. 2015). The requirement for ERα in the development and progression of these cancers is well established, and specific drug targeting of ERα has led to improved survival in breast cancer patients over the last 30 years (reviewed in Lumachi et al. 2015).

ID4 controls malignancy in BLBC

In contrast, the lineage dependencies of BLBC are poorly understood. Due to the developmental requirement for ID4, researchers hypothesised that ID4 may function in tumorigenesis. Studies focused on the importance of this protein in breast cancer have produced varied, complex results as a consequence of different models, technical approaches and experimental designs. As such, debate regarding the precise function of ID4 continues. ID4 effects varied outcomes in cell proliferation and differentiation depending on the availability of cofactors, and hence, play either a tumour suppressive or oncogenic function.

ID4 acts as a tumour suppressor, for example, in the normal prostate where it is highly expressed; this expression decreases in prostate cancer in a stage-dependent manner as a result of ID4 promoter hypermethylation (Carey et al. 2009, Sharma et al. 2012). High-grade tumours are associated with the absence of ID4 expression (Carey et al. 2009, Sharma et al. 2012). In this context, ID4 exerts anti-proliferative effects, in part, by increasing expression of the classical tumour suppressor genes p27 and p21 (Carey et al. 2009). This work highlights the divergent and context-dependent roles of ID4 in different hormone-dependent cancers.

There is now convincing evidence that ID4 is a proto-oncogene in BLBC. This subtype is associated with ID4 overexpression and amplification (de Candia et al. 2006, Turner et al. 2007, Natrajan et al. 2010). In BLBC, ID4 correlates with TP53 protein expression which associates with higher grade and risk of metastasis (Thike et al. 2015). ID4 is required for the proliferation of BLBC cell lines *in vitro* and *in vivo* and marks a subset of poor prognosis BLBCs (Junankar et al. 2015, Valenti et al. 2015).

ID4 and BRCA1 in BLBC

The relationship between ID4 and BRCA1 is poorly understood. The majority of the literature is based on

correlative data; however, emerging evidence is beginning to identify a fundamental genetic requirement for this interaction. The literature surrounding ID4 and BRCA1 is summarised in Table 1. ID4 and BRCA1 expression are inversely correlated in sporadic BLBC (Turner *et al.* 2007, Thike *et al.* 2015, Korlimarla *et al.* 2016). Beger and coworkers (2001) subsequently characterised the regulators of *BRCA1* gene expression and identified ID4 as an upstream regulator of the *BRCA1* promoter using an inverse genomics approach in luminal breast cancer cell lines. However, our research, and that from other laboratories, has shown exclusive ID4 protein expression in the ER α -negative subtypes of breast cancer, particularly BLBC where it is believed ID4 plays a crucial role in aetiology of this disease (de Candia *et al.* 2006, Roldán *et al.* 2006, Wen *et al.* 2012, Best *et al.* 2014, Junankar *et al.* 2015). Importantly, the inhibitory effect of ID4 on BRCA1 has since been confirmed in ER α -negative breast cancer cell lines (Crippa *et al.* 2014).

Further to ID4 directly controlling *BRCA1* gene expression, BRCA1 has been shown to reciprocally induce ID4 in normal kidney cells (Welch *et al.* 2002). It is currently unclear whether BRCA1 directly interacts with the ID4 promoter or whether this regulation occurs through other signalling pathways such as ER α . BRCA1 has been shown to inhibit ER α activity and activate transcription through the transcriptional regulator p300 (Fan *et al.* 1999), reviewed in Mullan *et al.* 2006). However, it is unclear how this may affect the regulation of ID4 in malignant breast tissue where ER α and BRCA1 expression is correlated (de Candia *et al.* 2006, Roldán *et al.* 2006). This suggests that ID4 may be exerting similar inhibitory effects on BRCA1 and ER α , and conversely that BRCA1 and ER α may demonstrate redundancy in inhibiting ID4.

Combined with the data above, this suggests that BRCA1 and ID4 are involved in a regulatory loop, which in normal cells maintains homeostasis: ID4 inducing proliferation and inhibiting differentiation (Shan *et al.* 2003, Fontemaggi *et al.* 2009) and BRCA1 promoting differentiation and preventing proliferation (Furuta *et al.* 2005). Supporting this model, depletion or mutation of *BRCA1* in mouse models results in the formation of aggressive tumours molecularly resembling BLBCs that interestingly express high levels of ID4 (Molyneux *et al.* 2010). Additionally, ID4 amplification is significantly associated with *BRCA1*-mutant BLBC, where it is amplified at more than twice the frequency compared with *BRCA1* wild-type BLBC (Prat *et al.* 2014; LA Baker, SA O'Toole,

C Selinger and A Swarbrick, unpublished observations). This indicates a genetic relationship between *ID4* and *BRCA1* and a selective advantage for genetic dysregulation of the *ID4* gene. Combined with the data presented above, we hypothesise that gain of ID4 is a mechanism through which the tumour suppressive function of BRCA1 can be inhibited.

ID4 and BRCAness

If ID4 regulates BRCA1, could this be important to the aetiology of BLBC? Could high ID4-expressing cancers identify patients with suppressed BRCA1 function?

The tumour suppressor BRCA1 is a crucial mediator of the DNA damage repair pathway (DDR). It repairs double-stranded DNA breaks and activates DNA damage-induced cell cycle checkpoints. In the absence of effective BRCA1 function, cells are unable to repair DNA, leading to the accumulation of mutations and the generation of genomic instability. Cells bypass normal checkpoint activation and progress aberrantly through the cell cycle (reviewed in Jasin 2002, Wu *et al.* 2010). *BRCA1*-mutant tumours demonstrate high genomic instability and increased frequency of rearrangements. The inability to effectively repair damaged DNA is characteristic of these tumours, a trait that is exploited with targeted therapies. Tumours with alterations to the *BRCA1*-homologous recombination (HR) pathway show clinical sensitivity to platinum-based chemotherapies (Tassone *et al.* 2009) and to targeted poly (ADP-ribose) polymerase (PARP) inhibitors (reviewed in Turner *et al.* 2004). PARP1 is a member of the DDR pathway that recruits and modifies nuclear proteins to respond to DNA damage (reviewed in Aly and Ganesan 2011). In the absence of effective PARP, cells are forced to repair damaged DNA through the *BRCA1*-mediated HR pathway. Hence, if PARP is inhibited in *BRCA1*-mutant breast cancers, DNA damage accumulates in the absence of effective repair mechanisms and cells undergo programmed cell death (Ashworth 2008). Hence, an important clinical opportunity exists for treatment of *BRCA1*-mutant tumours with available targeted therapeutics.

Evidence is emerging that some tumours can possess phenotypic similarities to *BRCA1*-mutant tumours without harbouring *BRCA1* mutations. This trait has been termed BRCAness (Turner *et al.* 2004). Tumours exhibiting BRCAness have a similar frequency of mutational and copy number aberrations compared with *BRCA1*-mutant BLBC tumours, indicating a similar defect

in HR. These tumours often possess epigenetic alterations to *BRCA1* (including promoter hypermethylation and thus gene inactivation (Catteau *et al.* 1999, Esteller *et al.* 2000)) and alterations in other DNA damage repair pathway proteins and thus resemble *BRCA1*-mutant BLBCs (reviewed in Turner *et al.* 2004). In some cases, this phenotype may be explained by loss of members of the *BRCA1*-HR pathway including BARD1 (a *BRCA1* protein interactor) (Sabatier *et al.* 2010), CHEK1 (cell cycle checkpoint mediator involved in mediating DNA repair), ATM (present at sites of DNA damage and recruits *BRCA1*), RAD51 (a functional marker of effective DDR in BLBC) (Lehmann *et al.* 2011), RAD17 (recruits MRE11-RAD50-NBS1 complex to regulate the DDR) and RAD50 (Wang 2007, Alli *et al.* 2009, Lehmann *et al.* 2011).

It has been suggested that BRCAness may associate with sensitivity to therapies traditionally targeting *BRCA1*-mutant patients (Turner *et al.* 2004, Silver *et al.* 2010, Konstantinopoulos *et al.* 2010). Identification of a new subset of patients responsive to these therapies is an exciting possibility for BLBC patients who have no access to effective targeted therapies (Lips *et al.* 2013). As described above, ID4 is highly associated with BLBC, inhibits *BRCA1* expression and is preferentially amplified in *BRCA1*-mutant BLBC. The expression and amplification status of ID4 in *BRCA1* heterozygote patients is currently unknown; however, we suggest that ID4 overexpression and amplification is acquired in *BRCA1* heterozygote mutant cancers or in wild-type patients to suppress remaining *BRCA1* function, resulting in *BRCA1*-mutant and BRCAness phenotypes. These combined data offer new insights into a potentially clinically important role for ID4 in these cancers. Indeed, platinum and PARP inhibitor sensitivity screens on breast cancer cell lines shows that ID4 high-expressing cell lines have increased sensitivity to these therapies ((Lehmann *et al.* 2011) and Laura Baker unpublished data). We suggest that high ID4 expression may mark a subset of BLBC patients (BRCAness and *BRCA1*-mutant patients) who may benefit from therapies traditionally used exclusively in *BRCA1*-mutant cancers (platinum-based therapies and PARP inhibitors) (Beger *et al.* 2001, Turner *et al.* 2007, Alli *et al.* 2009).

Conclusion

Characterisation of the important pathways controlling mammary gland development, and identification of the cells prone to transformation is crucial to furthering our understanding of mammary tumorigenesis. ID4, a well-established transcriptional regulator, controls

lineage commitment and terminal differentiation in the developing mammary gland. Loss of ID4 prevents normal ductal elongation and invasion of the mammary fat pad. Parallel to the role of ID4 in development, ID4 is emerging as a lineage commitment oncogene that is highly expressed and amplified in a subset of BLBC. Evidence suggests that BLBCs arise from luminal progenitor cells that often acquire mutation in or loss of *BRCA1*. The ensuing genomic instability may promote aberrant ID4 activity. These cells progress to form aggressive, poor prognosis ID4-positive tumours. ID4 positivity may mark a subset of BLBC patients who may respond to therapies traditionally used in *BRCA1*-mutant cancers including platinum-based chemotherapies and PARP inhibitors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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