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MINI-REVIEW

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'Omics driven discoveries of gene targets for apoptosis attenuation in CHO cells

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Abstract

Chinese hamster ovary (CHO) cells are widely used in biopharmaceutical production. Improvements to cell lines and bioprocesses are constantly being explored. One of the major limitations of CHO cell culture is that the cells undergo apoptosis, leading to rapid cell death, which impedes reaching high recombinant protein titres. While several genetic engineering strategies have been successfully employed to reduce apoptosis, there is still room to further enhance CHO cell lines performance. 'Omics analysis is a powerful tool to better understand different phenotypes and for the identification of gene targets for engineering. Here, we present a comprehensive review of previous CHO 'omics studies that revealed changes in the expression of apoptosis-related genes. We highlight targets for genetic engineering that have reduced, or have the potential to reduce, apoptosis or to increase cell proliferation in CHO cells, with the final aim of increasing productivity.

KEYWORDS

apoptosis, biopharmaceutical production, Chinese hamster ovary cells, genetic engineering, 'omics

1 | INTRODUCTION

The biopharmaceutical industry is fast growing, with global sales of over USD 188 billion in 2017 (Walsh, 2018). Major application areas include oncology and autoimmune/inflammatory disorders. Chinese hamster ovary cells (CHO) are the preferred production system for biopharmaceutical production due to their ability to perform post-translational modifications and their long term safety record and successful history of approval by regulatory bodies. These advantages are unlikely to change shortly (Walsh, 2018). As such, strategies for improving recombinant therapeutic protein titres are continuously being investigated with reports highlighting more than 100-fold increase in product titre, mainly due to media and process optimization (Datta, Linhardt, & Sharfstein, 2013; Wurm, 2004).

In contrast to the success in bioprocess optimization, cell line engineering for enhanced biopharmaceutical production has only

Abbreviations: Aip5 (Api5), apoptosis inhibitor 5; Alg-2, ALG2 α-1,3/1,6-mannosyltransferase; Anxa1, annexin A1; Bad, BCL2 associated agonist of cell death; Bak, BCL2 homologous antagonist killer; Bax, BCL2 associated X protein; Bcl10, BCL10 immune signaling adaptor; Bim (Bcl2l11), BCL2 like 11; Birc2, baculoviral IAP repeat-containing 2; Birc3, baculoviral IAP repeat-containing 3; Bnip2, BCL2 interacting protein 2; CHO, Chinese hamster ovary; Clu, clusterin; Cul2, cullin 2; Cul3, cullin 3; Ddah, dimethylarginine dimethylaminohydrolase; Ddit4, DNA-damage-inducible transcript 4; EpoFc, erythropoietin fusion protein; ER, endoplasmic reticulum; Fadd, Fas-associated death domain; FasL (Faslg), Fas ligand; Fastkd1, FAST kinase domains 1; Gclc, glutamate-cysteine ligase catalytic subunit; Gclm, glutamate-cysteine ligase modifier subunit; Gss, glutathione synthetase; Hsp27 (Hspb1), heat shock protein family B (small) member 1; Hsp70 (Hspa1a), heat shock protein family A (Hsp70) member 1A; Hspa8, heat shock protein family A (Hsp70) member 8; IFN-γ, interferon gamma; Lgals1, galectin 1; Madd, MAP-kinase activating death domain; Malt1, MALT1 paracaspase; Mcl1, MCL1 apoptosis regulator, BCL2 family member; Nod1, nucleotide-binding oligomerization domain containing 1; Nod2, nucleotide-binding oligomerization domain containing 2; Pasg (Hells), helicase, lymphoid specific; Pdcd4, programmed cell death 4; Pdcd6ip, programmed cell death 6 interacting protein; Prkdc, protein kinase, DNA activated, catalytic polypeptide; Rassf5, Ras association domain family member 5; Rnf216, ring finger protein 216; Tde1 (Serinc3), serine incorporator 3; Tgm2, transglutaminase 2; Tp53inp1, tumour protein p53 inducible nuclear protein 1; Tpt1, tumour protein, translationally-controlled 1; Xiap, X-linked inhibitor of apoptosis.

delivered modest improvements in productivity per cell. One of the few exceptions is the targeting of the apoptosis pathway. Programmed cell death, or apoptosis, is a suicide response from cells exposed to different stresses (nutrient limitation, accumulation of toxic by-products, hypoxia, and so forth; Arden & Betenbaugh, 2004). Logically, apoptosis is an undesirable feature for production cell lines as it results in a decrease in viable cell density and shorter culture duration, ultimately resulting in lower product titres (Fussenegger & Bailey, 1998). There are two pathways involved in initiating apoptosis, the extrinsic pathway, which senses extracellular stress signals, and the intrinsic pathway (endoplasmic reticulum- (ER) and mitochondria-mediated pathways), which senses intracellular stress signals, both reviewed in detail elsewhere (Elmore, 2007; Henry et al., 2020).

Many of the proteins involved in apoptosis pathways have been overexpressed (antiapoptotic proteins) or knocked-down (proapoptotic proteins) in CHO cells. Examples include the BCL-2 family proteins, caspases and heat shock proteins, with positive outcomes in delaying apoptosis and consequently improving production, as previously reviewed (Dietmair, Nielsen, & Timmins, 2011; Henry et al., 2020).

Modern rational cell design relies on 'omics strategies for the identification of new gene targets (Lewis, Abu-Absi, Borys, & Li, 2016). Various 'omics contrast studies have been performed in CHO cells to define features for improved biopharmaceutical production, as reviewed elsewhere (Dietmair, Nielsen, & Timmins, 2012; Stolfa et al., 2018). These studies have revealed changes in gene/protein expression belonging to diverse biological functions including protein synthesis, folding and secretion, transcription, cytoskeleton, cell cycle, and apoptosis. This review focuses on these 'omics datasets and describes apoptosis-related findings (Figure 1). Novel promising apoptosis gene targets for CHO cell engineering are highlighted (Table 1). This review is divided into studies that directly study apoptosis and the ones that focus on improving productivity.

2 | UNDERSTANDING APOPTOSIS: GROWTH PHASE COMPARISONS

To better understand apoptosis, noninduced apoptotic response of CHO cells has been studied at the transcriptome (Wong, Wong, Lee, et al., 2006), proteome (Wei et al., 2011) and metabolome (Chong et al., 2011) levels. Transcriptional profiling of apoptotic pathways in batch and fed-batch cultures of CHO cells producing recombinant human IFN-γ was studied. It was found that apoptosis occurred predominantly via the extrinsic death receptor- and mitochondria-mediated signalling pathways rather than the ER-mediated signalling pathway (Wong, Wong, Lee, et al., 2006). The authors found key early apoptosis signalling genes and proposed them as targets to delay apoptosis onset. These include *FasL*, *Fadd*, *Bim*, and *Bak* in batch cultures and *Fadd*, *Bim*, *Bad*, *Bax*, *Alg-2*, and *Requiem* in fed-batch cultures (Wong, Wong, Lee, et al., 2006). The overexpression of *Fadd* and the knock-down of *Alg-2* and *Requiem* (Wong, Wong, Nissom, Heng, & Yap, 2006) and the simultaneous knockout of *Bak* and *Bax* (Cost et al., 2010) were later proven to delay apoptosis and increase product titre.

On the other hand, when studying an IgG producer CHO cell line during prolonged batch cultivation, flow cytometry analyses of

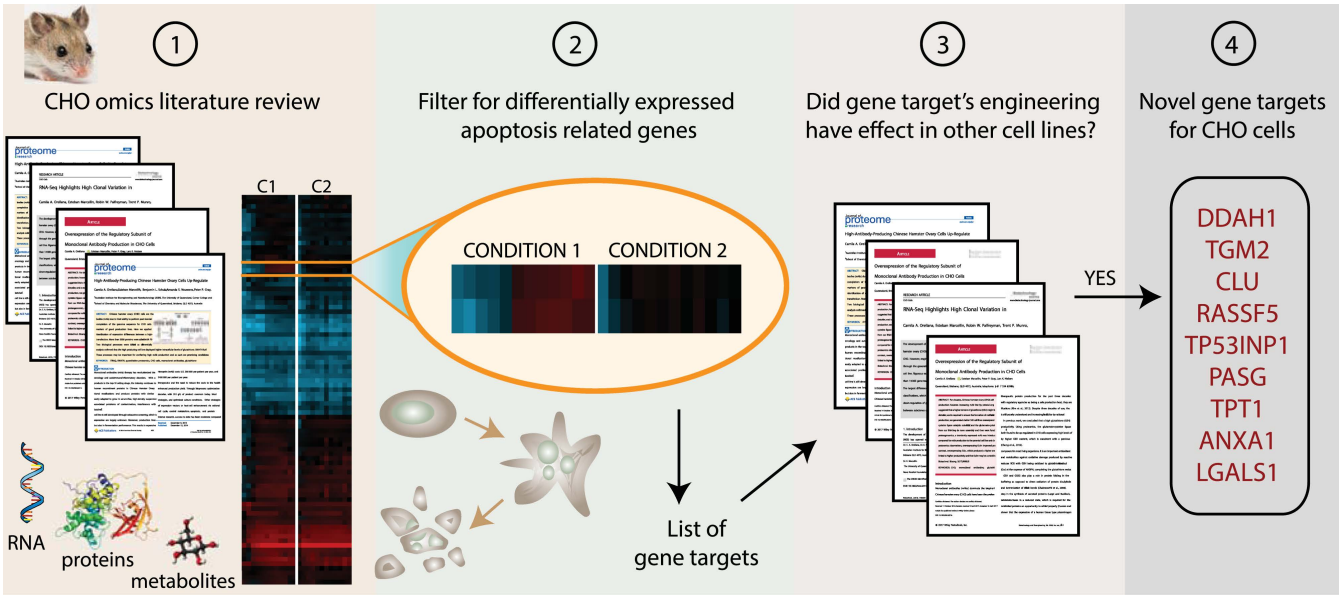

FIGURE 1 Workflow to find promising apoptosis targets for CHO cell engineering. (1) A comprehensive literature review of CHO omics studies was performed. (2) In each study, the list of differentially expressed transcripts or proteins was filtered for apoptosis-related genes. (3) The genes that have not been engineered in CHO cells were then searched for engineering strategies in other cell lines. (4) If positive outcomes were found in other cell lines, then the genes were listed as promising targets for CHO genetic engineering [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Gene targets with potential to reduce apoptosis or increase cell proliferation in CHO cells

Gene	Comparison	Regulation in CHO	Reference omics	Results in other cell lines	Reference other cell lines	Proposed engineering strategy in CHO
Ddah1 (antiapoptotic)	Metabolites, growth phases	Similar profile of dimethylarginine with caspases	Chong et al. (2011)	Overexpression promoted cell proliferation in human prostate cancer cell lines	Reddy et al. (2018)	Overexpression
Tgm2 (pro- and antiapoptotic)	Proteins, growth phases	Up in stationary phase	Carlage et al. (2012)	Downregulation decreased apoptosis in U937 and human neuroblastoma cells, and induced apoptosis in meningioma cells	Huang et al. (2014), Oliverio et al. (1999)	Downregulation. Overexpression can also be tested as results are cell line dependent
Clu (pro- and antiapoptotic)	Proteins, growth phases	Up in stationary phase	Carlage et al. (2012)	Downregulation triggered apoptosis and inhibited cell proliferation in HL-60 acute myeloid leukaemia cells. Overexpression inhibited H_2O_2-induced apoptosis in a mouse neuroblastoma cell line but induced apoptosis in both malignant and nonmalignant prostate epithelial cells	Scaltriti et al. (2004), Wang et al. (2015), You et al. (2003)	Overexpression. Downregulation can also be tested as results are cell line dependent
Rassf5	Transcripts, hyperosmotic stress	Up	Shen et al. (2010)	Overexpression suppressed cell proliferation and induced apoptosis in human osteosarcoma cell lines	Zhou et al. (2014)	Downregulation
Tp53inp1 (proapoptotic)	Transcripts, sodium butyrate treatment	Up	Birzele et al. (2010)	Downregulation increased the ability of hepatocellular carcinoma cells to form tumours	Ng et al. (2017)	Downregulation
Pasg (antiapoptotic)	Transcripts, sodium butyrate treatment	Up	Yee et al. (2008)	Deletion reduced cell proliferation and increased apoptosis in murine neural stem/progenitor cells	Y. Han, Ren, et al. (2017)	Overexpression
Tpt1 (antiapoptosis)	Transcripts, temperature shift to mild hypothermia	Down	Yee et al. (2009)	Overexpression prevented etoposide-induced apoptosis in HeLa and U2OS cells. Downregulation inhibited cell proliferation and induced apoptosis in human glioma cell lines	Jin et al. (2015), Li et al. (2001)	Overexpression
Anxa1 (proapoptotic)	Transcripts, high vs low producers	Up	Orellana et al. (2018)	Downregulation partially inhibited TRAIL-induced apoptosis in follicular undifferentiated	G Han, Lu, et al. (2017), Hsiang et al. (2006), Petrella et al. (2005)	Downregulation. Overexpression can also

(Continues)

TABLE 1 (Continued)

Gene	Comparison	Regulation in CHO	Reference omics	Results in other cell lines	Reference other cell lines	Proposed engineering strategy in CHO
				thyroid carcinoma cells. Overexpression induced apoptosis in prostate cancer cells while promoted proliferation of oesophageal squamous cell carcinoma cells		be tested as results are cell line dependent
Lgals1	Proteins and transcripts, high vs low producers	Up and down in the high producer clones	Carlage et al. (2009), Kumar et al. (2008), Meleady et al. (2008), Nissom et al. (2006), Orellana et al. (2015), Orellana et al. (2018)	Overexpression activated apoptosis in T cells and colorectal cancer cells. Upregulated in several cancer cells, promoting tumour cell growth	Astorgues-Xerri et al. (2014), Kovacs-Solyom et al. (2010), Perillo et al. (1995), Satelli and Rao (2011)	Downregulation. Overexpression can also be tested as results are cell line dependent
	Transcripts, temperature shift to mild hypothermia	Up after temperature shift				

various activated caspases demonstrated that the onset of apoptosis was primarily through the mitochondria and/or ER pathways (intrinsic), rather than the extrinsic apoptotic pathway (Wei et al., 2011). Proteomics analysis did not identify differentially expressed proteins that directly modulate apoptosis, although only 62 proteins were identified using differential gel electrophoresis. Major changes involved in the regulation of stress response and energy metabolism were hypothesised to help cope with the stress of nutrient depletion (Wei et al., 2011).

In fed-batch cultures, Chong et al. found a good correlation between some metabolites (nucleotides/nucleosides and amino acid derivatives) and caspases-3 and -7 activity (Chong et al., 2011). These metabolites were then added to CHO cell cultures in new media. Oxidized glutathione, adenosine 5′-monophosphate, guanosine 5′-monophosphate and dimethylarginine were shown to induce apoptosis. Following these results, the authors suggested targets for cell line engineering, which included the knock-down of ATP and adenosine receptors, overexpression of γ-glutamylcysteine synthetase (GCLC/GCLM) and glutathione synthetase to prevent glutathione depletion, and overexpression of dimethylarginine dimethylaminohydrolase to convert dimethylarginine into citrulline (Chong et al., 2011). *Gclm* overexpression was later proven to increase mAb titre in CHO cells by 70% (Orellana, Marcellin, Gray, & Nielsen, 2017). Overexpression of *Ddah1* (antiapoptotic) in human prostate cancer cell lines promoted cell proliferation by 28%–50% by degrading the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine, as well as increasing nitric oxide production (Reddy et al., 2018), looking promising as a target for CHO cell engineering (Table 1 and Figure 2).

Hernandez Bort et al. (2012) also characterized CHO mRNA and miRNA expression patterns during lag, exponential, and stationary phases. The authors found miRNAs upregulated during the exponential growth phase and/or downregulated in the stationary phase exhibiting functions linked to cell proliferation, cell cycle and apoptosis. Ten miRNAs were predicted to target mRNAs upregulated during stationary phase and whose transcription levels negatively correlate to their mRNA target levels. From these target mRNAs, 12 were related to programmed cell death, suggesting that the downregulation of miRNAs in late batch culture could be related to cell death. The miRNAs that target these apoptosis-associated genes were *miR15a*, *miR-16*, *miR-17*, *miR-27a* and *miR-30d*. The authors also suggested that the downregulation of the *miR-17-92* cluster in stationary phase could be involved in growth arrest (Hernandez Bort et al., 2012). The *miR-17*, *miR-92a* and *miR-17–92a* clusters were later overexpressed in CHO cells, and only *miR-17* overexpression enhanced growth and specific productivity, increasing EpoFc titre by three-fold (Jadhav et al., 2014).

Carlage et al. (2012) analysed the proteome of CHO cells overexpressing the antiapoptotic gene *Bcl-xL* during exponential and stationary phases and found that transglutaminase 2 (TGM2, pro- and antiapoptotic) and clusterin (CLU, pro- and antiapoptotic) were upregulated in the stationary phase (Carlage et al., 2012). TGM2 catalyses a Ca^{2+}-dependent crosslinking of lysine and glutamine

residues and also binds GTP (Fesus & Szondy, 2005). *Tgm2* downregulation in U937 (Oliverio, Amendola, Rodolfo, Spinedi, & Piacentini, 1999) and human neuroblastoma cells decreased apoptosis, while it induced apoptosis in meningioma cells (Huang et al., 2014). CLU is a glycoprotein implicated in several biological processes, including cell adhesion and apoptosis. Overexpression of *Clu* in a mouse neuroblastoma cell line (B103) inhibited H_2O_2-induced apoptosis (You, Ji, & Kwon, 2003) and its downregulation in HL-60 acute myeloid leukaemia cells triggered apoptosis and inhibited cell proliferation (Wang, Liu, Wang, Cai, & Zhang, 2015). However, its overexpression in both malignant and nonmalignant prostate epithelial cells induced apoptosis (Scaltriti et al., 2004). Even though contradictory results were observed for different cell lines, it is worthwhile testing TGM2 and CLU as targets for CHO cell engineering as they do have a strong effect in apoptosis (Table 1 and Figure 2).

Templeton et al. (2014) used ^{13}C fluxomics to understand central metabolism in cells overexpressing the engineered antiapoptotic gene Bcl-2Δ. The authors found that the reduced lactate accumulation observed in the engineered cell line was due to the redirection of pyruvate toward mitochondrial oxidation during the lactate-producing phase and an increase in lactate uptake rate during the lactate-consuming phase. These changes in flux were associated with the increase in biomass yield, peak viable cell density and integrated viable cell density observed in the Bcl-2Δ overexpressing cell line.

Overall, transcriptomics strategies have provided the greatest information about apoptosis, highlighting genes and mi-RNAs that have presented larger effects in enhancing CHO cell culture. However, it would be interesting to repeat the proteomics approach applying newer technologies such as tandem mass spectrometry, which nowadays is able to provide quantitative information for over 5000 proteins in CHO cells.

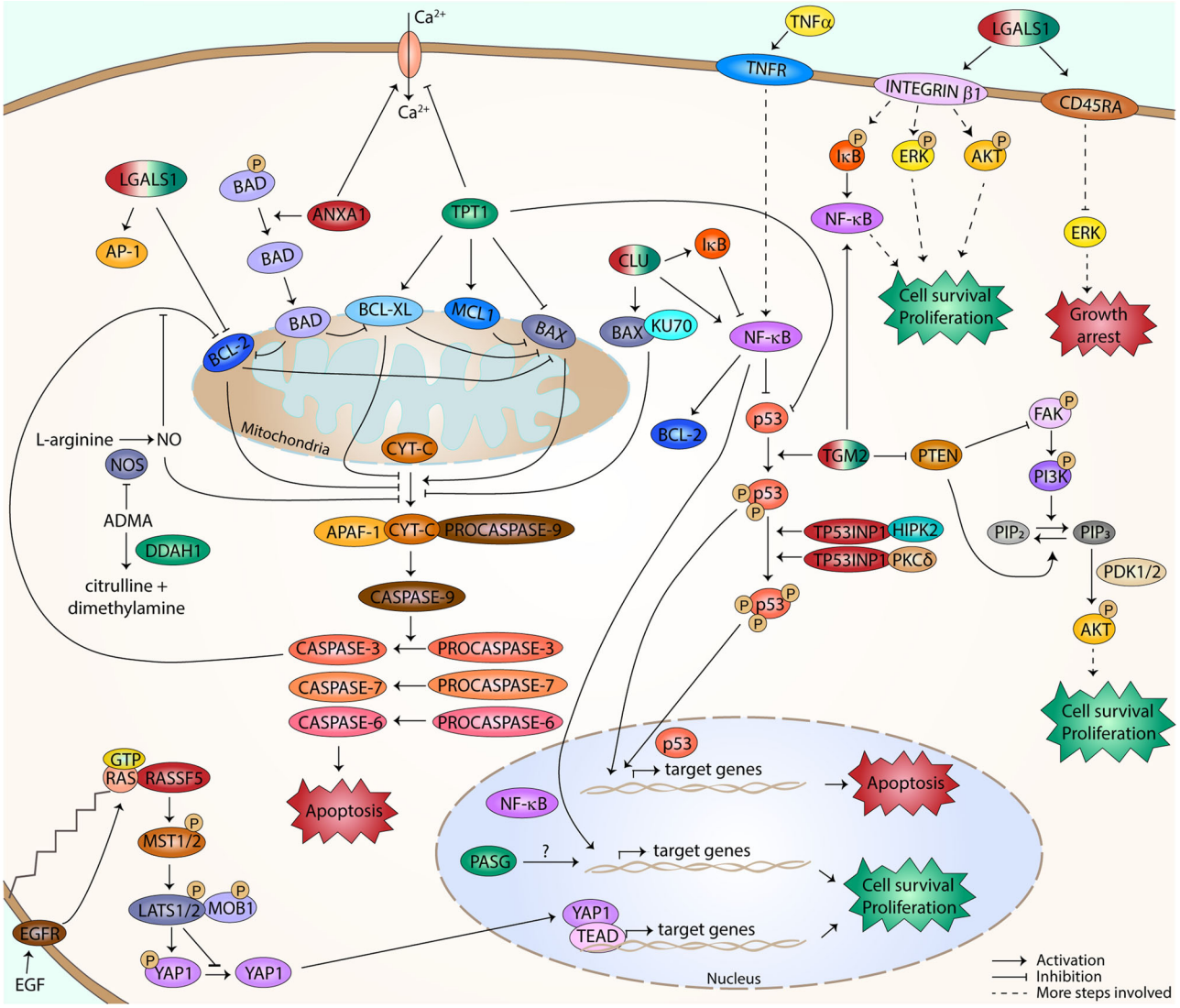

FIGURE 2 (See caption on next page)

3 | IMPROVING PRODUCTIVITY: CULTURE CONDITION COMPARISONS

Several studies have compared CHO cell lines under culture conditions that enhance specific productivity (q_p) in which apoptosis-related genes are differentially expressed with respect to controls. In most of these studies, the growth rate and integral viable cell density decreased when modifications were applied to the culture conditions, such as higher osmolarity, lower temperature shift or sodium butyrate addition. Nonetheless, the final titre remained similar or increased compared to the control, thus increasing q_p. Shen et al. found three apoptotic transcripts differentially expressed under hyperosmotic stress which repressed cell growth: *Malt1* downregulated and *Ddit4* and *Rassf5* upregulated (Shen et al., 2010), while Lee et al. did not find any apoptotic proteins de-regulated from the 54 identified proteins (Lee, Kim, Kim, & Lee, 2003) using two-dimensional gel electrophoresis. Ras association domain family member 5 (RASSF5) acts as a tumour suppressor, and it is downregulated in various cancer cell lines. Downregulation of *Rassf5* looks promising as a target for CHO cells (Table 1 and Figure 2) as its overexpression in human osteosarcoma cell lines suppressed cell proliferation and induced apoptosis (Zhou et al., 2014).

Under sodium butyrate treatment Yee et al. found that ten transcripts involved in apoptosis were differentially expressed: *Pdcd4* (downregulated), proapoptotic factor *Bnip2* (upregulated), antiapoptotic factors *Aip5*, *Pasg* and *Mcl1* (upregulated) and *Prkdc*, *Pdcd6ip*, *Tde1*, *Cul2* and *Cul3* (upregulated; Yee, de Leon Gatti, Philp, Yap, & Hu, 2008). Helicase, lymphoid-specific (PASG or HELLS) is a member of the SNF2 family of chromatin remodelling proteins, and high levels have been found in tumour cells (von Eyss et al., 2012). *Pasg* deletion in murine neural stem/progenitor cells reduced cell proliferation and increased apoptosis (Han et al., 2017) and its overexpression could be tested in CHO cells (Table 1 and Figure 2). Birzele et al. showed that the proapoptotic gene tumour protein p53 inducible nuclear protein 1 (*Tp53inp1*) was upregulated (Birzele et al., 2010) while De Leon et al. commented on an alteration of apoptosis-related genes but did not mention the gene names (De Leon Gatti, Wlaschin, Nissom, Yap, & Hu, 2007). Again, using two-dimensional

FIGURE 2 Overview of the role of the proposed protein targets on apoptosis/cell proliferation-related events. Many of these proteins have multifaceted functions, and only some of the proposed pathways in the literature are shown. Proteins targets in red are proapoptotic or induce growth arrest while protein targets in green are antiapoptotic or induce proliferation. (i) DDAH1 degrades asymmetric dimethylarginine (ADMA) which inhibits nitric oxide synthase (NOS) and thus, nitric oxide (NO) generation (De Gennaro Colonna et al., 2009). NO inhibits apoptosis by preventing the cleavage of the apoptosis suppressor B cell lymphoma gene 2 (BCL-2) and mitochondrial cytochrome-*c* (CYT-C) release. When released, CYT-C forms a complex with apoptotic protease-activating factor 1 (APAF-1) and pro-caspase-9, activating caspase-9, and subsequently executioner caspase-3, leading to apoptosis (Kim, Kim, Seol, Talanian, & Billiar, 1998). (ii) TGM2 activates p53 by phosphorylating the serine 15 and 20, reducing the ability of p53 to interact with MDM2, thus causing cell cycle arrest and apoptosis (Mishra & Murphy, 2006). However, TGM2 also promotes degradation of the tumour suppressor phosphatase and tensin homolog (PTEN) and results in constitutive activation of focal adhesion kinase (FAK)–phosphatidylinositol 3-kinase (PI3K)–protein kinase B (AKT) cell survival signalling (Verma et al., 2008). Furthermore, TGM2 induces constitutive activation of NF-κB, increasing cell proliferation (Mann et al., 2006). (iii) CLU inhibits apoptosis through stabilizing the association between KU70 and BCL-2 associated X protein (BAX), preventing mitochondrial cytochrome-*c* release. CLU also inhibits TNF-α-induced apoptosis by promoting TNF-α-mediated activation of nuclear factor κB (NF-κB) and BCL-2 overexpression. However, CLU may induce apoptosis by stabilizing inhibitory molecules (IκBs) that reduce NF-κB activity, thus upregulating p53 protein (Peng et al., 2019). (iv) Epidermal growth factor receptor (EGFR) activates RAS when an epidermal growth factor (EGF) signal is received. RASSF5 associates with RAS-GTP and phosphorylates and activates mammalian sterile 20- like kinase 1/2 (MST1/2) in the Hippo pathway. MSTG1/2 phosphorylates large tumour suppressor 1/2 (LATS1/2) and Mps one binder (MOB1), leading to phosphorylation of Yes associated protein 1 (YAP1) and thus its degradation. If not phosphorylated, YAP1 enters the nucleus, recruits the TEA domain (TEAD) family of transcriptional factors inducing gene transcription that contribute to cell proliferation (Liao, Jang, Tsai, Fushman, & Nussinov, 2017). (v) TP53INP1 forms complexes with the protein kinase homeodomain-interacting protein kinase-2 (HIPK2) or protein kinase C δ (PKCδ), phosphorylating p53 at serine 46. This leads to p53-target gene transcription (p53AIP1, p21, PIG3, and BAX), induction of G1 cell cycle arrest and increase in p53-mediated apoptosis (Shahbazi, Lock, & Liu, 2013). (vi) TPT1 inhibits p53-dependent apoptosis by binding and destabilizing p53 and repressing its transcription. TPT1 also bind and stabilize antiapoptotic BCL-2 family proteins, MCL1 and BCL-XL, which suppress apoptosis by binding and inactivating the proapoptotic proteins. Moreover, TPT1 blocks the formation of proapoptotic BCL-2 family protein BAX homodimers, required for its apoptotic activity. It also inhibits apoptosis induced by Ca^{2+} influx by binding to Ca^{2+}. High Ca^{2+} intracellular level injure mitochondrial membranes leading to the release of CYT-C and apoptosis-inducing factor (AIF), resulting in apoptosis (Nagano-Ito & Ichikawa, 2012). (vii) ANXA1 induces the dephosphorylation of the member of the BCL-2 family, BAD, which can then translocate to the mitochondria, heterodimerize with BCL-2 or BCL-XL and promote apoptosis. Furthermore, ANXA1 activates Ca^{2+} influxes in a concentration-dependent manner, leading to apoptosis (Solito et al., 2003). (viii) LGALS1 binds to β1-integrin increasing the phosphorylation of extracellular signal-regulated kinases (ERK), protein kinase B (AKT) and inhibitor of kB (IkB), inducing proliferation. The phosphorylation of IκB induces NF-κB activation suppressing the apoptotic pathways. However, LGALS1 also binds to a protein tyrosine phosphatase receptor type C (CD45RA) inducing growth arrest or inhibition of the progression of the cell cycle by inducing suppression of ERK phosphorylation (Abroun et al., 2008). LGALS1 also stimulates activating protein-1 (AP-1) and downregulates BCL-2 by inhibiting concanavalin A induction of BCL-2 protein, inducing apoptosis (Rabinovich et al., 2000). (ix) PASG is required to maintain proper DNA methylation and gene expression patterns that are required for normal growth and longevity. The specific role remains unclear, however, PASG knockout upregulates tumour suppressor genes such as $p16^{INK4a}$, $p19^{ARF}$, p53, and p21 and downregulates BMI-1 (a negative regulator of $p16^{INK4a}$; Sun et al., 2004) [Color figure can be viewed at wileyonlinelibrary.com]

electrophoresis, a subsequent investigation was unable to find apoptotic proteins to be de-regulated in the 28 proteins identified (Baik, Joo, Kim, & Lee, 2008). The knockdown of the tumour suppressor *Tp53inp1* in hepatocellular carcinoma cells (MHCC97L) increased the ability of cells to form tumours in the liver (Ng et al., 2017) and it could be used as a target to increase cell proliferation (Table 1 and Figure 2).

In studies involving a temperature shift to mild hypothermia, the antiapoptosis related transcript *Tpt1* was downregulated (Yee, Gerdtzen, & Hu, 2009), the antiapoptotic HSPA8 protein was upregulated (Baik et al., 2006), importin-α was downregulated and LGALS1 protein was upregulated (Kumar, Gammell, Meleady, Henry, & Clynes, 2008) after the temperature shift. Overexpression of tumour protein, translationally-controlled 1 (*Tpt1*) prevented HeLa cells and U2OS cells from undergoing etoposide-induced apoptosis (Li, Zhang, & Fujise, 2001) and its downregulation in human glioma cell lines (U251) inhibited cell proliferation and induced apoptosis (Jin et al., 2015). These results indicate that it is worthwhile testing *Tpt1* in CHO cells (Table 1 and Figure 2).

The proposed genes, if engineered in CHO cells, are likely to improve or restore the reduced growth seen in cultures with operational conditions such as hyperosmolarity, temperature shift or addition of sodium butyrate. However, it is unclear whether the engineered cells will keep the higher specific productivity observed under these conditions.

4 | IMPROVING PRODUCTIVITY: CHO CELL LINES COMPARISONS

Other authors have compared CHO cell lines with different specific productivities (q_p), and again apoptosis-related genes have been found to be differentially regulated. Chen et al. (2017) found the negative apoptosis regulators *Madd*, *Birc2* and *Birc3*, the positive apoptosis regulator *Nod1* and the apoptotic genes *Fastkd1* and *Rnf216* downregulated in the CHO-DG44 high producer cell line. Orellana et al. (2018) found 90 apoptosis regulator transcripts upregulated in the CHO-K1 high producer cell line, including the negative regulators *Clu*, *Gclm*, *Hsp27 (Hspb1)*, *Hsp70 (Hspa1a)*, *Nod2*, *Xiap* and *Birc3*, the positive regulators *Anxa1* and *Nod1*, and the *Lgals1* gene. Discrepancies between both studies may arise from differences in the cell lines, product, media and culture conditions used. Annexin A1 (ANXA1) is involved in diverse biological functions such as in inflammatory pathways, cell proliferation and regulation of apoptosis, amongst others (Lim & Pervaiz, 2007). Overexpression of *Anxa1* induced apoptosis in prostate cancer cells (Hsiang, Tunoda, Whang, Tyson, & Ornstein, 2006), while its downregulation partially inhibited TRAIL (tumour necrosis factor-related apoptosis-inducing ligand)-induced apoptosis in follicular undifferentiated thyroid carcinoma cells (Petrella et al., 2005). However, the overexpression of *Anxa1* also promoted proliferation of oesophageal squamous cell carcinoma cells (Han et al., 2017) and both, overexpression and knockdown strategies could be tested in CHO cells (Table 1 and Figure 2).

Orellana et al. (2015) also found Galectin 1 (LGALS1) protein upregulated in the CHO-K1 high producer clone while three other proteomics studies found that LGALS1 was downregulated in the CHO-DG44 and CHO-DXB11 high producer clones (Carlage et al., 2009; Meleady et al., 2008; Nissom et al., 2006). LGALS1 is a member of the family of β-galactoside binding proteins and is known to activate apoptosis in T cells (Kovacs-Solyom et al., 2010; Perillo, Pace, Seilhamer, & Baum, 1995) and colorectal cancer cells (Satelli & Rao, 2011), and its downregulation seems a promising strategy for CHO genetic engineering. However, LGALS1 is also known to be upregulated in several cancer cells, promoting tumour cell growth (Astorgues-Xerri et al., 2014), so its downregulation might inhibit cell proliferation and survival, and its overexpression should also be tested (Table 1 and Figure 2).

Nissom et al. (2006) also found Bcl10 pro-apoptosis transcript downregulated in the CHO-DXB11 high producer clone, while Meleady et al. (2008) showed that the apoptosis inhibitor HSP27 was upregulated in the CHO-DXB11 high producer clone, which has already been shown to reduce apoptosis and increase titre when overexpressed (Tan et al., 2015).

Finally, a ^{13}C fluxomics analysis showed that the CHO-K1 high producing clones produce less lactate, have an elevated citric acid cycle flux and direct a greater amount of pyruvate toward mitochondrial metabolism compared to low- or nonproducing cell lines (Templeton et al., 2017). Furthermore, clones with apoptosis-resistant features (Bcl-2Δ overexpression) simultaneously increased antibody production and glucose metabolism (hexokinase and phosphofructokinase fluxes).

Relevant genes that have shown to alter apoptosis and or proliferation have been discussed here and coluld be targets for CHO cell culture enhancement in the future.

5 | CONCLUSIONS

'Omics approaches have contributed to the understanding of apoptosis in CHO cells and have led to the identification of propitious targets for rational genetic engineering. Differential expression analysis helps pinpoint nontrivial gene targets, which otherwise would be difficult to identify. In CHO cells, various apoptosis-associated genes were found to be de-regulated in phenotypes of interest, such as increased productivity. Specifically, apoptosis regulators whose functions in apoptosis are not yet completely understood, appear as differentially expressed. The overexpression or downregulation of these targets have successfully reduced apoptosis or increased cell proliferation in various cancer cells, and are consequently promising targets for CHO cell engineering. Some of these gene targets have shown contradictory results for different cancer cells. Therefore both overexpression and downregulation should be tested.

Most of the CHO 'omics studies to date have been simple contrasts with a single type of 'omics, analysed using standard *t* test statistics. The depth and power of 'omics studies can be increased by

using a multi-omics approach combing transcriptomics, proteomics, metabolomics, and fluxomics and exploiting network topology when analysing the data. Furthermore, to best exploit the continuous creation of 'omics datasets in CHO cells, uniform conditions across a broad set of cell lines should be considered to find universal gene targets. This review shows that there is still room for improvements of CHO cell lines for biopharmaceutical production and the use of 'omics studies for gene target elucidation offers unprecedented opportunities to positively impact final product titre.

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AUTHOR CONTRIBUTIONS

Camila A. Orellana conceived and designed the manuscript, collected and analysed the data and drafted and edited the manuscript. Verónica S. Martínez, Michael A. MacDonald and Matthew N. Henry conceived, designed, critically revised and edited the manuscript. Marianne Gillard, Peter P. Gray, Lars K. Nielsen, Stephen Mahler and Esteban Marcellin critically revised and edited the manuscript.

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