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Bioactive Lipids in Stem Cell Differentiation

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1. Introduction

Bioactive lipids are lipids with cell signaling functions. In the last two decades, they have become increasingly important in many fields of biology. They are the main diffusible mediators of inflammatory responses in tissues and regulate the polarity of cellular membranes. They are also critical for cell fate decisions during stem cell differentiation by inducing apoptosis or sustaining cell survival and polarity. The bioactive lipids discussed here belong to the classes of phospho- and sphingolipids. Mainly three different types of lipids and their function in stem cell differentiation will be reviewed in detail: phophatidylinositols (PIPs), lysophospholipids and eicosanoids, and the sphingolipid ceramide and its derivative sphingosine-1-phosphate (S1P).

2. Biological Function of bioactive lipids in stem cell differentiation

2.1 Phosphatidylinositols

The phosphatidylinositols PI(3,4)P2 and PI(3,4,5)P3 generated by class I phosphatidyinositol-3-kinase (PI3K) upon induction of tyrosine receptor kinases or G-protein coupled receptors (GPCRs) are known to be the major activators of the Akt/PKB cell signaling pathway for cell survival and differentiation (Callihan et al., 2011; Frebel &Wiese, 2006; Layden et al., 2010; Paling et al., 2004; Storm et al., 2007; Umehara et al., 2007). The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid phosphatase that catalyzes the hydrolysis of PIP3 to PIP2, which leads to inactivation of the Akt/PKB cell signaling pathway and loss of pluripotency in stem cells (Groszer et al., 2001; Korkaya et al., 2009; Otaegi et al., 2006). PTEN is a tumor suppressor mutated in many types of cancer and it is critical for the controlled growth of embryonic tissue and ES cells.

PTEN converts PIP3 into PIP2 (Fig. 1). Since PIP3 activates the Akt/PKB cell signaling pathway, thus PTEN catalyzing PIP3 hydrolysis is a negative regulator of Akt/PKB. Consistent with this function, deletion of PTEN activates Akt/PKB-dependent cell signaling pathways (Groszer et al., 2001). PTEN mutations are often found in human cancers such as glioblastoma, prostate cancer, and breast cancer. Loss of function of this tumor suppressor gene results in the up-regulation of the Akt/PKB-to-β-catenin pathway (Fig. 2A) (Korkaya et al., 2009). Akt/PKB phosphorylates and therefore, inactivates glycogen synthase-3β (GSK-3β), a protein kinase in the Wnt signaling pathway that phosphorylates β-catenin (Doble &Woodgett, 2003; Ikeda et al., 2000; van Noort et al., 2002). The oncogene β-catenin is an important adhesion protein and transcription factor for genes involved in proliferation. When phosphorylated by GSK-3β, β-catenin (in a protein complex with adenomatous

polyposis coli or APC) is proteolytically degraded and thus, adhesion lost and proliferation reduced. Consistent with this function, deletion of β-catenin results in loss of pluripotency and early embryonic death of the respective knockout mouse (Haegel et al., 1995). Likewise, deletion of PTEN results in increased β-catenin levels and increased pluripotency or malignancy (Groszer et al., 2001). Therefore, the PTEN vs. PI3K-to-Akt/PKB antagonism is interesting in two biological contexts with respect to stem cell differentiation: maintenance of pluripotent stem cells and tumorigenesis of cancer stem cells. In the first context, inhibition of PTEN, activation of PI3K and Akt/PKB, or inhibition of GSK-3β will be useful to maintain pluripotent ES cells. In the second context, activation of PTEN, inhibition of PI3K and Akt/PKB, or activation of GSK-3β may be a useful strategy to eliminate cancer stem cells.

In the cultivation process of ES cells, elevated expression of the transcription factors Oct-4 and Nanog is essential for maintenance of pluripotency (Bhattacharya et al., 2003; Sato et al., 2004). It has been shown that two cell signaling pathways are critical for this regulation: the janus kinase/signal transducer and activator of transcription 3 (Jak/Stat3) and the Akt/PKB signaling pathways (Fig. 2A) (Kelly et al., 2011; Paling et al., 2004). In the cultivation of mouse ES cells, the most important growth factor activating Stat3 and Akt/PKB is LIF (leukemia inhibitory factor), an interleukin 6 class cytokine binding to LIF receptor α (LIFRα) (Cartwright et al., 2005; Niwa et al., 1998; Okita &Yamanaka, 2006; Schuringa et al., 2002; Takao et al., 2007). In vitro, LIF is added to the medium when cultivating undifferentiated mouse ES cells on feeder fibroblasts and in feeder-free culture. In vivo, LIF is generated by the trophoectoderm from where it penetrates the inner cell mass, the source of pluripotent ES cells in the pre-implantation embryo. In human ES cells, the role of LIF as "guardian" of pluripotency is taken over by fibroblast growth factor (FGF) (Lanner &Rossant, 2010; Li et al., 2007) (Fig. 2A). Binding of FGF-2 to the FGF receptor 2 (FGFR2) activates similar cell signaling pathways in human ES cells as stimulated by LIF in mouse ES cells: Jak/Stat3, mitogen-activated protein kinase (MAPK), and Akt/PKB (Lanner &Rossant, 2010; Li et al., 2007). However, FGFR-dependent signaling is very diverse and it depends on individual receptor protein complexes which specific response is elicited by FGF. For example, in mouse ES cells, FGF-2 is used to maintain the multipotent neuroprogenitor stage and to prevent further neuronal differentiation. In human ES cells, supplementation of the serum-free cell culture medium with FGF-2 is critical to prevent apoptosis and to maintain pluripotency.

The role of lipids as the key factors in the PI3K-to-Akt/PKB-to-β-catenin cell signaling pathway is obvious since phosphatidylinositols (PIPs) are lipids by provenance. Unfortunately, PIPs are not applicable as exogenous factors that can be simply added to stem cell media since these lipids are part of an intracellular cell signaling cascade not easily accessible to the outside of the cell. However, there are other lipid-regulated pathways that are dependent on the activation of cell surface receptors, which is of tremendous advantage if one attempts to use lipids as exogenously added growth or differentiation factors (see section 2.2). The two receptors involved in maintenance of pluripotency, LIFRα and FGFR2 are both tyrosine receptor kinases which are not directly activated by lipids, although indirect regulation by so-called "lipid rafts" has been discussed (see section 2.4) (Lee et al., 2010b; Yanagisawa et al., 2004b, 2005).

In addition to using natural lipids as ligands, stem cell differentiation can also be modulated by pharmacologic reagents that are either lipid analogs, inhibitors of enzymes in lipid

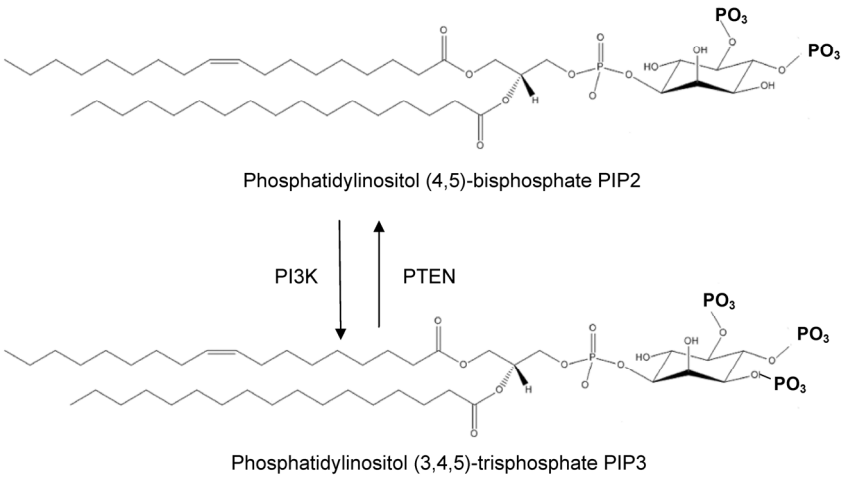

Fig. 1. Metabolism of phosphatidylinositols in the PI3K-to-Akt/PKB cells signaling pathway for ES cell pluripotency. PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10

metabolism, or drugs targeting downstream effectors of lipid-regulated cell signaling pathways. Two drugs that are inhibitors of protein kinases in the LIFRα and FGFR2 pathways have been tested on their effect on pluripotency: LY294002 and indirubin-3-monoxime, two inhibitors specific for PI3K and GSK-3β, respectively (Chen et al., 2006; Chen et al., 2000; Ding &Schultz, 2004; Ding et al., 2003; Lyssiotis et al., 2011; Otaegi et al., 2006; Paling et al., 2004; Sato et al., 2004). The PI3K inhibitor LY294002 has been shown to reduce the capacity of mouse and human ES cells to self-renew and to undergo subsequent steps of lineage specification and differentiation (Paling et al., 2004). These effects are likely to involve differentiation stage-specific (contextual) other cell signaling pathways downstream (or parallel) to the PI3K-to-Akt/PKB signaling axis. While it may not be desired to interfere with ES cell pluripotency, LY294002 and other PI3K and Akt/PKB inhibitors are currently tested for cancer treatment, in particular for targeting cancer stem cells (Bleau et al., 2009; Plo et al., 1999). If one desires to sustain self-renewal of ES cells, GSK-3β inhibitors such as indirubin-3-monoxime or BIO are attractive candidates. BIO has been successfully used to maintain pluripotency in human ES cells (Sato et al., 2004). Additional effectors targeting GSK-3β are synthetic agonists of the Wnt receptor Frizzled, however, their use in stem cell differentiation is not yet sufficiently investigated (Lyssiotis et al., 2011).

Interestingly, inhibitors of the mitogen activated protein kinase (MAPK) pathway such as the MAPK kinase (MEK) inhibitor PD98059 have been used with mouse ES cells to promote self-renewal or pluripotency (Buehr &Smith, 2003; Li et al., 2007). This appears paradoxical since LIFRα as well as FGFR2 are known to activate MAPK, which suggests that activation of MAPK is involved in pluripotency. However, only transient MAPK activation to promote G1 re-entry is useful for self-renewal while prolonged activation will promote differentiation (Fig. 2B). Therefore, a combination of LIF with the MAPK-kinase (MEK) inhibitor PD95059 activating PI3K-to-Akt/PKB while inhibiting MAPK signaling has been successfully used to promote pluripotency in mouse ES cells, but also to enhance the

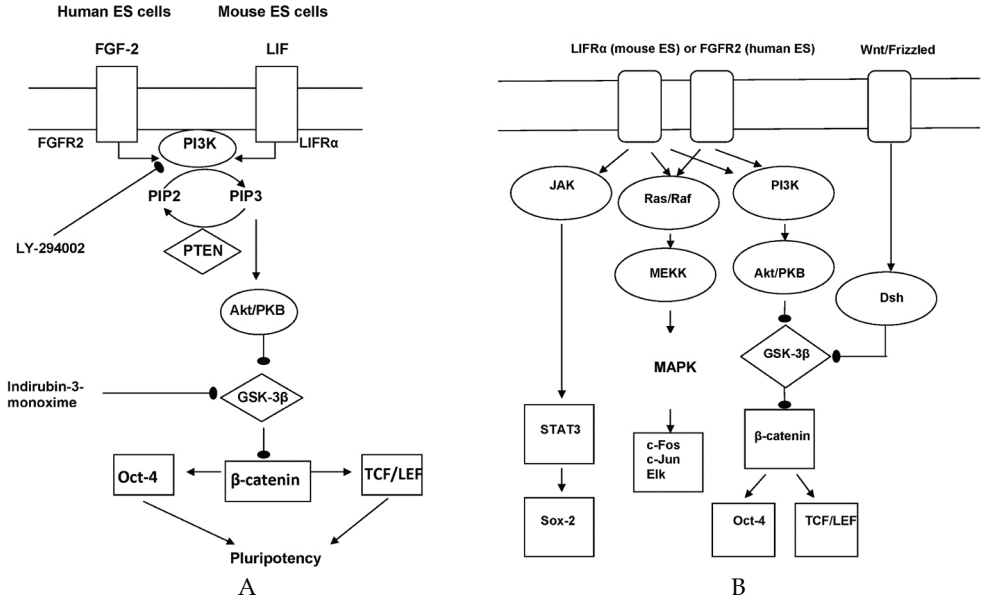

Fig. 2. Cell signaling pathways for ES cell pluripotency. Elliptic circles label enzymes that promote pluripotency, while diamonds label enzymes that reduce pluripotency and promote differentiation. MAPK shows both, pro-pluripotency or pro-differentiation activity in human or mouse ES cells, respectively.

generation of induced pluripotent stem (iPS) cells (Li et al., 2007; Lyssiotis et al., 2011). The situation in human ES cells, however, is different. In contrast to mouse ES cells, inhibition of the MAPK cell signaling pathway reduces the potential of undifferentiated human ES cells to self-renew, indicating that FGFR2-mediated activation of Ras/Raf-to-MEK-to MAPK is critical for human ES cell pluripotency (Ding et al., 2010). A similar role has been found for Bmp4, which promotes pluripotency in mouse and differentiation in human ES cells (Bouhon et al., 2005; Zeng et al., 2004; Zhang et al., 2010). It is quite possible that this difference depends on which other pathways for pluripotency are co-activated such as Jak/Stat3 in mouse or Activin in human ES cells. Bioactive lipids are important in that they co-regulate several cell signaling pathways critical for pluripotency and differentiation of ES cells, in particular MAPK and PI3K downstream of ClassA/Rhodopsin-like GPCRs, which will be discussed in the next section.

2.2 Lysophospholipids and eicosanoids

Lysophospholipids (LPLs) are lipids generated by hydrolytic cleavage of fatty acid from glycerophospholipids, which is catalyzed by phospholipases. Distinct phospholipases cleave off either one of the two (PLA1 and PLA2) or both (PLB) fatty acid residues, or they cleave off the phosphate-containing head group (PLC) or the alcohol (PLD) (Gardell et al., 2006; Hla et al., 2001; Hla et al., 2000; Lin et al., 2010; Meyer zu Heringdorf &Jakobs, 2007; Okudaira et al., 2010; Radeff-Huang et al., 2004; Tigyi &Parrill, 2003; Ye et al., 2002). PLA2 generates arachidonic acid, the precursor for the generation of eicosanoids, a group of inflammatory mediators including prostaglandins and leukotrienes (Funk, 2001; Jenkins et

al., 2009; Khanapure et al., 2007; Lambeau &Gelb, 2008; Szefel et al., 2011; Wymann &Schneiter, 2008). Similar to the PLD reaction, lysophospholipase D or autotaxin generates lysophosphatidic acid (LPA) from lysophosphatidylcholine (Nakanaga et al., 2010; Okudaira et al., 2010; Samadi et al., 2011). LPA receptors are critical in cell proliferation and tumorigenesis and have recently been shown to promote proliferation of human neural precursor cells (Callihan et al., 2011; Hurst et al., 2008; Lin et al., 2010; Pebay et al., 2007; Pebay et al., 2005; Pitson &Pebay, 2009).

Arachidonic acid, generated by PLA2 from phospholipids such as phosphatidylcholine (Fig. 3A) is converted to a variety of pro-inflammotory eicosanoids among which prostaglandins, thromboxanes, and leukotrienes are the most important signaling lipids (Fig. 3B). The effect of eicosanoids on ES cells is not well understood and research is mostly limited to results with mouse ES cells. Interestingly, lysophospholipids such as LPA and eicosanoids such as prostaglandin E2 (PGE2) appear to activate similar downstream cell signaling pathways, mainly the PI3K-to-Akt/PKB, MAPK, and Wnt/GSK-3β pathways (Callihan et al., 2011; Goessling et al., 2009; Logan et al., 2007; North et al., 2007; Pebay et al., 2007; Pitson &Pebay, 2009; Yun et al., 2009). In contrast to LIFRα or FGFR2, however, stimulation of Akt/PKB by PGE2 has not been reported to sustain pluripotency, but is rather anti-apoptotic/cell protective and promotes stem cell proliferation. This may not be surprising since generation and conversion of arachidonic acid is often a response to hypoxic insults, which can damage mitochondria and induce apoptosis. Notably, inhibition of eicosanoid biosynthesis reduces the potential of mouse and human ES cells to self-renew, indicating a role of eicosanoids in stem cell maintenance or pluripotency (Yanes et al., 2010). Thromboxane has not been described to play a role in stem cell differentiation, maybe because its main function is rather confined to platelet aggregation. In contrast, prostacyclin, a similar eicosanoid in platelet aggregation has been shown to promote cardiogenic differentiation from human ES

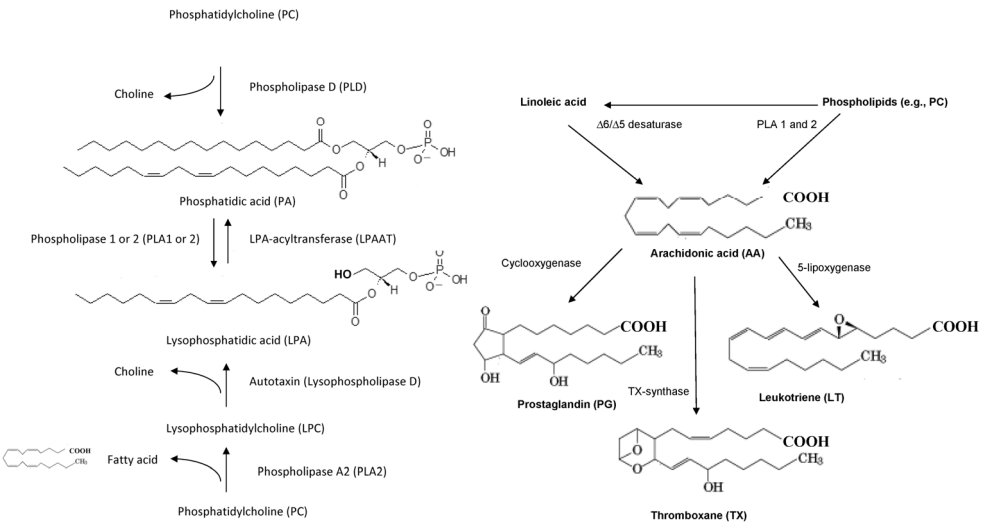

Fig. 3. Biosynthesis pathways in lysoposphatidic acid (LPA) and eicosanoid metabolism

cells (Chillar et al., 2010; Xu et al., 2008). In addition to prostacyclin, leukotriene of the LTD4 type has been used in several studies to promote proliferation and cardiovascular differentiation of mouse ES cells (Finkensieper et al., 2010; Funk, 2001; Kim et al., 2010).
The effect of prostaglandins and other eicosanoids on ES cells is worth discussing in an important aspect of human health care. Inhibitors of cyclooxygenase 2 (Cox-2), the enzyme critical for PGE2 production, are taken by nearly everyone to ease up head ache, back pain, and inflammation. The Cox-2 inhibitor aspirin is one of the most successfully administered drugs world-wide. A recent study on the negative effect of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin on the differentiation of human ES cells suggests that one has to be careful with the use of NSAIDs when human ES cells are to be transplanted for heart tissue repair (Chillar et al., 2010). These observations suggest that eicosanoids are important in cardiogenic/cardiovascular differentiation of ES cells.
The eicosanoid as well as lysophospholipid receptors belong to the family of Class A Rhodopsin-like GPCRs (Callihan et al., 2011; Hla et al., 2001; Kostenis, 2004; Lin et al., 2010; Pitson &Pebay, 2009; Radeff-Huang et al., 2004). They mediate the activation of downstream cell signaling pathways through different types of GTPases, mainly Gi, Gq, and G12/13,

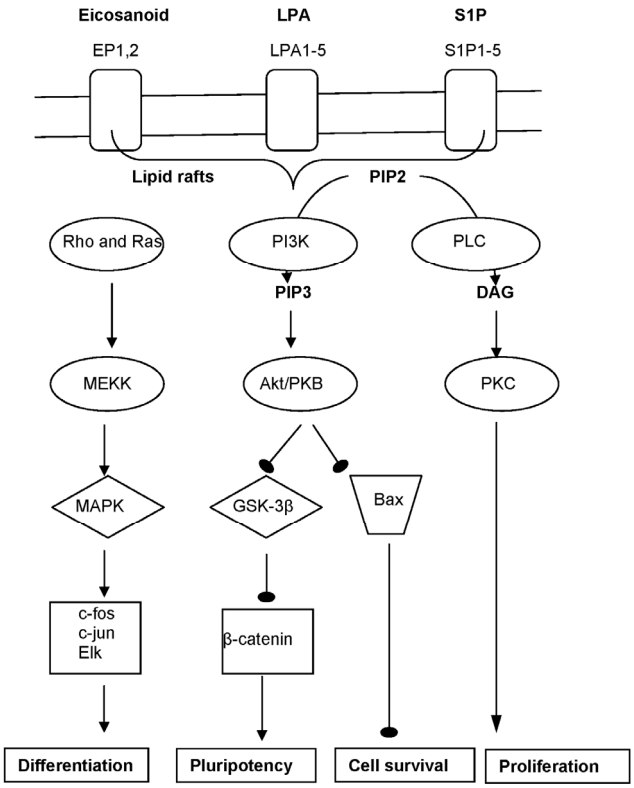

Fig. 4. GPCR-dependent cell signaling pathways with similar function for ES cell pluripotency and differentiation. DAG, diacylglycerol; PLC, phospholipase C; EP, eicosanoid receptor.

acting upon PI3K-to-Akt/PKB (Gi), Ras-to-ERK (Gi, Gq) Rho (G12/13), and PLC-to-PKC (Gq) cell signaling pathways for pluripotency and cell survival (Akt/PKB), proliferation (Rho and PKC), and differentiation/specification (MAPK) pathways (Fig. 4). Hence, combinations of particular cell signaling lipids with cytokines or growth factors such as LIF or FGF-2 activating similar effectors have been found to be useful in directing stem cell fate toward pluripotency, proliferation, or differentiation, respectively (Hurst et al., 2008; Kilkenny et al., 2003; Layden et al., 2010; Pebay et al., 2007; Radeff-Huang et al., 2004). There are five GPCRs for each LPA and sphingosine-1-phosphate (S1P) expressed in mouse and human ES cells.

2.3 Ceramide and sphingosine-1-phosphate

Sphingolipids are acyl (fatty acid) derivatives of the amino alcohol sphingosine. They encompass sphingosine, ceramide, and ceramide derivatives such as sphingomyelin, ceramide-1-phosphate, S1P, and glycosphingolipids (Fig. 5A for structures) (Bartke &Hannun, 2009; Chalfant &Spiegel, 2005; Chen et al., 2010; Futerman &Hannun, 2004; Hannun et al., 2001; Hannun &Obeid, 2002, 2008; Lebman &Spiegel, 2008; Merrill et al., 1997; Spiegel &Milstien, 2003; Strub et al., 2010; Takabe et al., 2008). Important biological functions of sphingolipids are cell signaling for inflammation, apoptosis, cell cycle regulation, and autophagy (Bartke &Hannun, 2009; Basu &Kolesnick, 1998; Bieberich, 2004, 2008a; Futerman &Hannun, 2004; Gulbins &Kolesnick, 2003; Haimovitz-Friedman et al., 1997; Hannun &Obeid, 2008; Morales et al., 2007). Most recently, particular sphingolipids have also been implicated in ES cell differentiation and cell polarity (Bieberich, 2004, 2008a, b, 2010; Bieberich et al., 2003; Bieberich et al., 2001; Bieberich et al., 2004; Gardell et al., 2006; Goldman et al., 1984; Harada et al., 2004; Hurst et al., 2008; Jung et al., 2009; Pebay et al., 2007; Pebay et al., 2005; Pitson &Pebay, 2009; Salli et al., 2009; Walter et al., 2007; Wang et al., 2008a; Wong et al., 2007; Yanagisawa et al., 2004a). Ceramide has been shown to induce apoptosis specifically in residual pluripotent stem (rPS) cells that cause teratomas (stem cell-derived tumors) after stem cell transplantation. S1P has been found to promote oligodendrocyte differentiation (see section 3.2. for discussion).

Ceramide is the precursor of all bioactive sphingolipids. It is synthesized in three different metabolic pathways. Figure 5B shows that sphingolipid metabolism is integrated into phospholipid (i.e., PC), one carbon unit (i.e., choline), fatty acid (i.e., palmitoyl CoA for de novo biosynthesis and other fatty acids in the salvage pathway), and amino acid (i.e., serine in de novo biosynthesis) metabolism (Bartke &Hannun, 2009; Bieberich, 2004, 2008a; Chen et al., 2010; Futerman &Hannun, 2004; Futerman &Riezman, 2005; Gault et al., 2010; Hannun et al., 2001; Luberto &Hannun, 1999). In cell cultures, plenty of these precursors are provided in the medium, which may not necessarily reproduce the metabolic situation of stem cells or other cell types in vivo. Recently, our group has found that neural crest-derived stem or progenitor cells are sensitive to alcohol due to ethanol-induced elevation of ceramide and induction of apoptosis (Wang &Bieberich, 2010). Apoptosis can be prevented by supplementing the medium with CDP-choline, This effect can be explained by providing excess of substrate required to drive conversion of ceramide to SM using the interconnection of the Kennedy pathway for phospholipid biosynthesis and the SM cycle (Fig. 5B). Choline can also be replenished from the one carbon unit metabolism, which establishes the interconnection of sphingolipid metabolism with this metabolic pathway.

The fatty acid metabolism interconnects with sphingolipid biosynthesis twice, in the de novo and salvage pathways. The de novo pathway uses palmitoyl CoA and serine for a

condensation reaction that is the first step in ceramide biosynthesis. Since serine is used as the second substrate, de novo biosynthesis ties into the amino acid metabolism as well. The salvage pathway uses a variety of activated fatty acids for re-attachment to sphingosine (Fig. 5B). While supply with precursors for lipid metabolism may not be critical in vitro, specialized tissues or cells such as astrocytes providing nutrients and metabolic precursors to neurons or neural stem cells in vivo maybe more sensitive toward lipid imbalances as observed in fetal alcohol syndrome and Alzheimer's disease (Adibhatla &Hatcher, 2008; Cutler et al., 2004; De Vito et al., 2000; Hirabayashi &Furuya, 2008; Jana et al., 2009; Jana &Pahan, 2010; Muscoli et al., 2010; Riboni et al., 2002; Satoi et al., 2005; Wang et al., 2008b). In particular, neural stem cells are confined to distinct morphological cell complexes which tightly control the interaction with other cells and therefore, comprise "metabolic niches" that may control supply with metabolic precursors and lipid cell signaling factors.

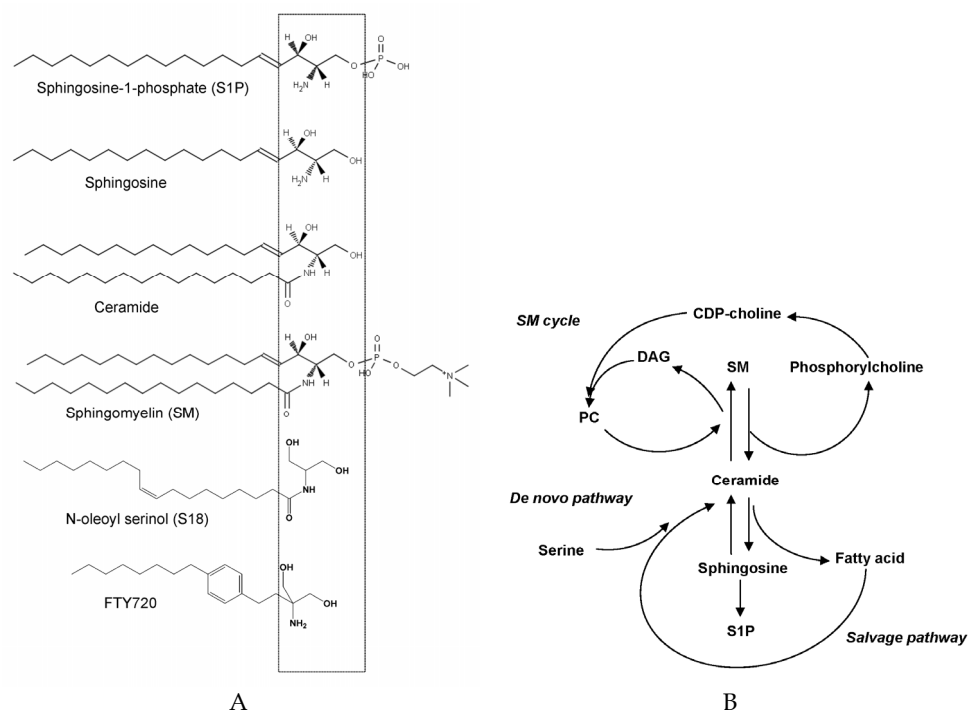

Fig. 5. Structures of ceramide precursors and derivatives with cell signaling function and interconnection of ceramide metabolism with other lipid and amino acid metabolism. N-oleoyl serinol (S18) or FTY720 are analogues of ceramide or S1P, respectively. Box shows common structural motif.

Regulation of sphingolipid metabolism by its interconnection with other lipid metabolic pathways has a direct impact on lipid-dependent cell signaling. Ceramide is the precursor of S1P, which is a ligand for five distinct S1P receptors (S1P1-5) on the cell surface and also binding partner/co-factor for at least three intracellular proteins, histone deacetylase 1 and 2

(HDAC 1 and 2) in the nucleus, the E3 ubiquitin ligase TRAF2, and prohibitin in the mitochondria (Alvarez et al., 2010; Callihan et al., 2011; Hait et al., 2009; Hait et al., 2006; Hurst et al., 2008; Pitson &Pebay, 2009; Radeff-Huang et al., 2004; Sanchez &Hla, 2004; Spiegel &Kolesnick, 2002; Strub et al., 2011) . The effect of intracellular S1P on stem cell differentiation is not known. However, there is solid experimental evidence that S1P has profound effects on ES cells and ES cell-derived neural progenitors via S1P receptors, which will be discussed in section 3.2.

2.4 Terpenoids, sterols, glycosphingolipids, and lipid rafts

The previous sections discussed bioactive lipids that are known to act through lipid receptors or binding proteins. There are many more lipids that regulate cell signaling pathways through a mechanism known as "lipid rafts" or "lipid microdomains" (Bieberich, 2008a; Lee et al., 2010b; Lingwood &Simons, 2010; Miljan &Bremer, 2002; Ohanian &Ohanian, 2001; Yanagisawa et al., 2005). Lipid rafts are areas in the cell membrane (or intracellular membranes) that emerge from the self-assembly of lipids in an ordered structure. They are believed to show high affinity to specific cell signaling proteins such as growth factor or cytokine receptors, which leads to clustering and activation of these receptors. Therefore, bioactive lipids can affect stem cell differentiation in two different ways: direct interaction with lipid receptors such as GPCRs and lipid raft-dependent activation of growth factor or cytokine receptors such as LIFRα or FGFR2 (Bieberich, 2008a; Bryant et al., 2009; Gutierrez &Brandan, 2010; Lee et al., 2010b; Yanagisawa et al., 2005). Lipids that form rafts are sphingomyelin, cholesterol, and glycosphingolipids. In addition, signal transduction proteins such as Ras can be modified with fatty acids (palmitoylation) or terpenoids (farnesylation, geranylation) and glycophosphatidylinositol (GPI anchor), which tremendously increases membrane binding and raft association (Levental et al., 2010; Lingwood &Simons, 2010; Resh, 2004; Roy et al., 2005). It has been shown that particular glycosphingolipids termed gangliosides can regulate ES cell differentiation by the activation of FGFR2 and other receptors in lipid rafts (Bieberich, 2004; Yanagisawa et al., 2005). An example for this mechanism is the corrective activity of the ganglioside GM1 on the effect of the fungus toxin fumonisin B1, which causes neural tube defects by inhibiting sphingolipid biosynthesis (Gelineau-van Waes et al., 2005; Marasas et al., 2004). It has also been demonstrated that the activity of sonic hedgehog, a morphogen critical for germ layer formation is functionally dependent on palmitoylation and modification with cholesterol (Gofflot et al., 2003; Guy, 2000; Incardona &Roelink, 2000; Karpen et al., 2001; Kelley et al., 1996; Lewis et al., 2001; Li et al., 2006). Inhibition of cholesterol biosynthesis with statins leads to aberrant embryo development. Although these are impressive examples of the effect of lipid modification and lipid raft formation on stem cell differentiation and embryo development, it is presently not known how to specifically utilize this mechanism in controlling the differentiation of ES cells. It is also not clear, which differentiation potential cholesterol has besides being critical for lipid raft formation. There is a plethora of steroid hormones such as estrogen or progesterone that are bioactive lipids activating nuclear receptors critical for embryo development. Progesterone is a particularly curious case since it is added to most of the supplements (e.g., N2, B27) found in defined media used for the in vitro maintenance and differentiation of ES cells. The use of this and other bioactive and synthetic lipids for the in vitro differentiation of ES cells will be discussed in the following section.

3. Bioactive lipids and their use for in vitro differentiation of embryonic stem cells

3.1 Induction of apoptosis in teratoma-forming stem cells by ceramide analogs

The reliability and safety of current stem cell differentiation protocols is still a matter of controversy. Many studies have shown that even when using similar protocols for the in vitro differentiation of ES cells, transplantation can lead to the formation of teratomas (Baker, 2009; Bieberich, 2008b; Blum &Benvenisty, 2008; Fong et al., 2010; Fujikawa et al., 2005; Lee et al., 2009; Vogel, 2005; Wang et al., 2010). Teratomas are stem cell-derived tumors that are fatal if they grow in the brain or heart. Teratomas may arise from any type of pluripotent cells, including induced pluripotent stem (iPS) cells. Therefore, they are a major safety concern, in particular when using larger numbers of ES or iPS cell-derived cells. Our studies have shown that teratomas arise from a particular type of residual pluripotent stem (rPS) cells that maintain the expression of the pluripotency transcription factor Oct-4 and fail to differentiate or undergo apoptosis (Bieberich, 2008a, b, 2010; Bieberich et al., 2003; Bieberich et al., 2004). However, we have also found that they co-express prostate apoptosis response 4 (PAR-4), a protein that sensitizes cells toward ceramide-induced apoptosis. Using a water-soluble ceramide analog termed N-oleoyl serinol or S18, which was for the first time synthesized in our laboratory, we were able to rid stem cells grafts of teratoma-forming rPS cells (Bieberich et al., 2002; Bieberich et al., 2000). We have shown that S18 promotes binding of atypical PKC (aPKC) to PAR-4, which inhibits the aPKC-activated NF-κB cell survival pathway and induces apoptosis in rPS cells (Bieberich, 2008a; Krishnamurthy et al., 2007; Wang et al., 2009; Wang et al., 2005). These cells are eliminated because they are sensitive to S18. Neural progenitor cells will survive and undergo further differentiation because they show no or only low level expression of PAR-4.

3.2 Induction of oligodendrocyte differentiation by S1P and S1P analogs

It has been shown that S1P and the S1P prodrug analog FTY720 promote cell survival and differentiation of primary cultures of oligodendroglial precursor cells (OPCs) (Bieberich, 2010; Coelho et al., 2010; Jung et al., 2007; Miron et al., 2008a; Saini et al., 2005). We have found that teratoma-forming rPS cells do not express the S1P receptor S1P1, which makes them vulnerable to ceramide or S18-induced apoptosis (Bieberich, 2008b, 2010; Bieberich et al., 2004). In contrast, ES cell-derived neural progenitor cells express S1P1. Our studies have shown that in the presence of S18 and FTY720, neural progenitor cells will survive and undergo oligodendroglial differentiation because they are insensitive to S18 (PAR-4 is not expressed). At the same time, OPC differentiation is promoted by FTY720 or S1P (S1P1 is expressed). Implantation of S18 and FTY720-treated neural progenitors does not result in teratoma formation and leads to integration of the grafted cells into highly myelinated areas of the brain such as the corpus callosum (Bieberich, 2010). Therefore, a combined treatment with ceramide analogs and S1P analogs or S1P receptor agonists is a promising strategy to control ES cell differentiation toward OPCs that are useful for treatment of de- or dysmelination diseases such as multiple sclerosis. Interestingly, the addition of S1P analogs to the ceramide analog S18 resulted in a shift of predominantly neuronal differentiation (as promoted by S18 alone) of ES cells toward oligodendroglial lineage, which is an impressive example for the impact of bioactive lipids on stem cell differentiation.

3.3 Synthetic lipids as small molecular effectors for ES cell differentiation

The use of defined media supplemented with small molecule effectors that control the in vitro differentiation of stem cells is a promising strategy to generate transplantable progenitor cells that have not been in contact with animal-derived products such as serum. Currently, there are more than twenty compounds available that specifically induce differentiation of ES cells toward progenitors of bone, heart, muscle, or brain tissue. Although most of these compounds are not considered bioactive lipids because they are synthetic drugs not found in biological organisms, almost all of them are lipids with respect to their chemical structure. One of the first synthetic lipids used a small molecule effector for ES cell differentiation is a bioactive lipid with critical function in brain development: retinoic acid (Dinsmore et al., 1996; Guan et al., 2001; Hu et al., 2009; Jiang et al., 2010; Lee et al., 2010a; Liour et al., 2006; Mayer-Proschel et al., 1997; Mummery et al., 1990; Murashov et al., 2005; Osakada &Takahashi, 2011; Plachta et al., 2004). Mouse and human ES cells respond to a brief exposure to retinoic acid by accelerating differentiation into motoneurons, interneurons, and even oligendendrocytes when combined with specific growth factors such as FGF-2 or platelet-derived growth factor (PDGF). Another bioactive lipid used for in vitro differentiation of mouse and human ES cells, in particular toward oligodendroglial lineage is thyroid hormone (T3) (Glaser et al., 2007; Kang et al., 2007).

In addition to these naturally occurring lipids, synthetic lipids have been isolated from chemical libraries using various bioassays for ES cell differentiation. Indirubin-3-oxime type compounds for maintenance of pluripotency have already been discussed in section 1. A more detailed discussion of these small molecule effectors can be found in the following articles (Ding et al., 2003; Lyssiotis et al., 2011; Zhu et al., 2010). The advantage of these compounds emerges from their lipid-like structure, which allows for penetration of the blood brain barrier. Provided that toxicity issues do not prevent the use of these compounds in vivo, bioactive and synthetic lipids are likely to develop into powerful pharmacologic drugs that can be used for in vitro differentiation of ES or iPS cells and then after transplantation, for further treatment of the patient to enhance the in vivo differentiation of the grafted cells. One of the first drugs with this dual potential of in vitro and in vivo use is FTY720 (Bieberich, 2010; Coelho et al., 2007; Lee et al., 2010a; Miron et al., 2008a; Miron et al., 2008b; Napoli, 2000). It is quite expectable that many of these "dual use" drugs will play an important role in the clinical application of ES and iPS cells.

4. Conclusions and perspectives

The goal of this chapter was to review current knowledge on bioactive lipids in embryonic stem cell differentiation. One of the important results of this analysis is the insight into the interconnection between lipid metabolism and signaling function. Unlike most proteins, lipids can be converted into derivatives that either complement or antagonize each other's cell signaling function. For example, conversion of PC to LPA and eisosanoids has similar effects on enhancing pluripotency. On the other hand, conversion of ceramide to S1P can have opposite functions, in particular with respect to apoptosis and survival of pluripotent stem cells. Another important insight is that most bioactive lipids cooperate with cytokine and growth factor receptors providing the possibility to combine these factors with the respective lipids in defined media for controlled stem cell differentiation. For example, FGF-2 can be combined with the ceramide analog S18 to promote neuronal differentiation, or with S18 and FTY720 to enhance specification to oligodendroglial lineage. This provides the

opportunity to generate bioactive lipids or lipid analogs that can be applied for in vitro differentiation of stem cells and then for further treatment of the patient who has received the stem cell graft. These "dual use" bioactive lipids will be of tremendous value for the therapeutic application of stem cells.

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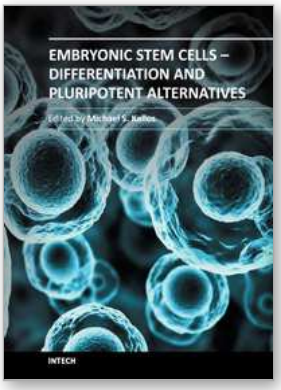

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The ultimate clinical implementation of embryonic stem cells will require methods and protocols to turn these unspecialized cells into the fully functioning cell types found in a wide variety of tissues and organs. In order to achieve this, it is necessary to clearly understand the signals and cues that direct embryonic stem cell differentiation. This book provides a snapshot of current research on the differentiation of embryonic stem cells to a wide variety of cell types, including neural, cardiac, endothelial, osteogenic, and hepatic cells. In addition, induced pluripotent stem cells and other pluripotent stem cell sources are described. The book will serve as a valuable resource for engineers, scientists, and clinicians as well as students in a wide range of disciplines.

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