Stem Cell Rev
DOI 10.1007/s12015-007-0004-8

Signaling Pathways in Cancer and Embryonic Stem Cells

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Abstract Cancer cells have the ability to divide indefinitely and spread to different parts of the body during metastasis. Embryonic stem cells can self-renew and, through differentiation to somatic cells, provide the building blocks of the human body. Embryonic stem cells offer tremendous opportunities for regenerative medicine and serve as an excellent model system to study early human development. Many of the molecular mechanism underlying tumorigenesis in cancer and self-renewal in stem cells have been elucidated in the past decade. Here we present a systematic analysis of seven major signaling pathways implicated in both cancer and stem cells. We present on overview of the JAK/STAT, Notch, MAPK/ERK, PI3K/AKT, NF-kB, Wnt and TGF-β pathways and analyze their activation status in the context of cancer and stem cells. We focus on their role in stem cell self-renewal and development and identify key molecules, whose aberrant expression has been associated with malignant phenotypes. We conclude by presenting a map of the signaling networks involved in cancer and embryonic stem cells.

Keywords Stem cells · Cancer · Signaling pathways · JAK/STAT · Notch · MAPK/ERK · PI3K/AKT · NF-κB · Wnt · TGF-β

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Introduction

It is estimated that our bodies consist of about 200 different somatic cell types, which have a limited proliferative capacity. In vitro, somatic cells undergo 50–60 population doublings before entering a non-reversible growth arrest, termed senescence [1, 2]. In contrast, stem cells and cancer cells bypass this replicative barrier, and acquire the ability to divide indefinitely, when grown under specific conditions in vitro [3].

Three types of stem cells with different potency have been so far recognized: Embryonic stem cells, chord blood/placental stem cells and adult stem cells. Among these categories, embryonic stem cells have been shown to be totipotent: the ability to give rise to all the 200 different somatic cells as well as germ cells [4, 5]. Stem cells are characterized by their ability to self-renew and differentiate into various organs derived from the three embryonic germ layers: endoderm, mesoderm and ectoderm. As a consequence, embryonic stem cells offer a unique opportunity to study fundamental processes of human development and hold great promise to provide therapeutic interventions for regenerative diseases. Stem cells from umbilical cord and placental tissue have also been reported. While their potential to give rise to different cells types is still under scrutiny, it is believed that they behave as multipotent cells in a manner similar to adult stem cells. Finally, adult stem cells represent a rare population of multipotent cells in the adult body. The best example of adult stem cells are the hematopoeitic population of the bone marrow which can contribute to a number of lineages, as well as maintaining a niche of undifferentiated pluripotent cells. The differentiation potential of adult stem cells is more limited, yet they play a crucial role in regulating tissue homeostasis, neural plasticity and maintenance as well as regeneration of organs after injury.

The genetic elements that are necessary for tumorigenesis have been well characterized in the past 20 years. Human somatic cells can become cancerous by activation of defined genetic elements such as introduction of oncogenic RAS, blockage of the p53/Rb pathway and activation of telomerase [6]. Several other well-described alterations are necessary to initiate metastasis and angiogenesis [3, 7]. However, considering the generally low mutation rate of somatic cells and their relatively short replicative lifespan, one caveat of the this multi-step model is how a somatic cell can acquires all these "essential" alterations [8].

Recently, an elegant model proposed that cancers might arise from cells that replicate more extensively in the human body: the Cancer Stem Cells (CSC). Although the origin of CSC remains unclear, it is possible that CSC could arise from adult stem or progenitor cells that, due to their pivotal role in tissue homeostasis, have higher capacity to proliferate and are more prone to accumulate mutations over the course of their lifespan. CSC are generally defined as subpopulation of cells within a tumor that have the ability to initiate, regenerate and sustain tumors within xenograft mouse models [9]. Serial transplantation of CSC gave rise to cancers, which reconstituted the tissue-specific heterogeneous cell types of the parental tumor, reflecting the differentiation capabilities of CSC. Thus, CSC can self-renew and produce differentiated progenitors that constitute the bulk of the tumor. There is currently however no described link between CSC and metastasis, a gap in our knowledge that is currently under scrutiny.

The CSC hypothesis suggests that cancers contain of a small subpopulation of CSC that initiate and sustain tumor growth and metastasis. This hypothesis has two major implications: first, tumorigenesis requires multiple mutations; it is conceivable that they are more likely to occur in long-lived cells such as adult stem cells. Secondly, if proven to be correct, this hypothesis might have dramatic implications for cancer therapy [10, 11]. The success of conventional cancer therapeutics has been measured by their ability to shrink the bulk tumor mass. However, these drugs might not target the small subpopulation of CSC that fuel and re-initiate tumor growth. Consistent with this notion, tumors often reappear, even after initially successful treatment, (as measured by tumor shrinkage). In contrast, therapeutics that target the CSC population offer the potential to eradicate the cancer. It is therefore crucial to identify CSC, to study their characteristics on a molecular level and to investigate their propensity to manipulate their environment.

Cancers and embryonic stem cells both have the capability to undergo rapid clonal proliferation. In cancer, proliferation is uncontrolled and detrimental, whereas during human development, bursts of clonal proliferation are highly concerted and controlled. Furthermore, similarly to metastatic cancer cells, stem cells have the ability to inhabit and thrive in various environments of the human body. Considering these intriguing similarities and the implications they might have on designing therapeutic intervention, the comparison of the molecular pathways involved in tumorigenesis, self-renewal and differentiation in cancer cells as well as cancer stem cells has become a priority.

In this review we focus on signaling pathways that have been shown to be implicated in both cancer cells and stem cells. From the nine main signaling pathways involved in embryonic development and cancer, seven of them have been implicated in both cancer and stem cells [12]. These are: the JAK/STAT pathway, NOTCH signaling pathway, the MAP-Kinase/ERK pathway, the PI3K/AKT pathway, the NFkB pathway, the Wnt pathway and the TGFβ pathways (Fig. 1). We will discuss what is currently known about the role of each pathway in both tumor cells and stem cells, addressing issues of necessity versus sufficiency, as well as their relationship with the cancer stem cell hypothesis. This will allow us to identify current gaps in our knowledge and define the high priority questions that still await an answer.

The JAK/STAT Pathway

Cells can communicate with each other through the secretion of cytokines, small (8–30 kDa) soluble proteins. To date, roughly 20 cytokines have been identified. Upon binding their cognate receptors, receptor-associated Janus Kinases (JAKS) phosphorylate tyrosine residues of the ligand-bound receptors, as well as interacting Signal Transducers and Activators of Transcription (STATs). Tyrosine phosphorylated STATs form homodimers, shuttle to the nucleus and participate in transcriptional regulation of a variety of genes [13, 14]. STATs are also activated in response to growth promoting factors such as Epidermal Growth Factor (EGF) or Platelet-Derived Growth Factor (PDGF) [13]. Thus, the JAK/STAT pathway plays an important role in mediating cell fates, such as apoptosis, differentiation and proliferation, in response to growth promoting factors and cytokines.

The JAK/STAT Pathway in Cancer

The JAK/STAT pathways is intimately linked to growth factor signaling, apoptosis and the cellular immune response. Deregulated JAK/STAT signaling can contribute directly and indirectly to tumorigenesis [15]. Mutations, fusions, and/or amplification of JAK/STAT signaling components, such as the HER2/neu- in mammary and stomach carcinomas, or Epidermal Growth Factor-Receptor

(EGF-R) in breast, brain and stomach tumors, can confer hypersensitivity to mitogenic signals and promote proliferation [16, 17]. Furthermore, STAT3 is constitutively activated in several major human carcinomas and some hematologic tumors. Notably, STAT3 is persistently active in over 50% of lung and breast tumors and more than 95% of head and neck cancers [14]. As mentioned above, JAK/STAT signaling also mediates the cellular response to cytokines; impaired STAT signaling could therefore also indirectly contribute to tumor formation by compromising tumor immune surveillance.

The JAK/STAT Pathway in mESC Self Renewal

The JAK/STAT pathway can also play a role in maintaining the pluripotent state of mouse embryonic stem cells (mESC). During in vitro culture of mESC, the Leukemia Inhibitory Factor (LIF) can substitute for the presence of a feeder cell layer. LIF binds to the cytokine receptor gp130, which in response leads to tyrosine phosphorylation of STAT3. mESC, grown in the presence of LIF and serum phosphorylate STAT3 and remain pluripotent. However, depletion of gp130, STAT3 or LIF does not affect pluripotency in mESC. Furthermore, mice deficient for components of the LIF/STAT3 pathway exhibit no stem cell defects [18]. Taken together these results suggest that signaling through STAT3 might be sufficient but not necessary to ensure pluripotency in mESC. In contrast, LIF does not support pluripotency of hESC when grown in the absence of feeder cells and STAT1, 3 and 5 are not phosphorylated in pluripotent hESC [19, 20]. Consistent with these results, addition of LIF to the hESC culture medium is not sufficient to maintain pluripotency. This apparent contradiction between mouse and human ESC suggests that the pathways that maintain pluripotency in the two species are different.

Notch Signaling

The Notch signaling pathway consists of a membrane-tethered protein, that upon ligand binding undergoes proteolytic cleavage and releases a transcription factor that shuttles to the nucleus [12]. Notch signaling is involved in tissue homeostasis and development in metazoan animals. During tissue development, Notch signaling plays an integral part in mediating signaling between adjacent cells. As an example, Notch can inhibit the spread of cellular differentiation within a tissue (lateral inhibition) or promote adjacent cells to adopt the same cell fate (lateral induction) [21]. Activation of this pathway is triggered by the interaction of membrane-associated Delta, Serrata or Lag2 Notch ligands with their cognate Notch receptor. Ligand binding induces a series of proteolytic cleavages within the receptor, generated by ADAM-type metalloproteases and γ-secretase, that ultimately liberate the intracellular domain of Notch (NICD). The extracellular product (Notch EC) remains associated with the transmembrane product (Notch-TM), whereas the released NICD shuttles to the nucleus where it forms a transcriptional activation complex with the DNA-binding factors CSL (CBF1/Suppressor of Hairless / Lag1) and the Mastermind-like protein family (MAML).

The Role of Notch Signaling in Cancer

T-cell acute lymphoblastic leukemia / lymphoma (T-ALL) is a blood cancer that results from unrestrained proliferation of immature T-cells [22]. Mutations in the Notch signaling pathway has been identified in ~55–60% of human T-ALL, yet it remains unclear whether these mutations initiated tumorigenesis, or whether they were acquired as a secondary event during continued selection for aggressive proliferation. At least in mouse models, Notch plays a crucial role in the earliest stages of T-cell development and is dispensable for further differentiation to intrathymic T-cells. These results suggest that neoplasias could occur when Notch signaling remains active beyond normal development [23]. Recent studies have implicated aberrant Notch signaling in human breast tumors, melanoma progression, medullablastoma and ovarian cancers. In the case of human breast tumors, amplification of Notch receptors and the presence of ligands, such as Jagged 1 correlate with a more aggressive disease phenotype. This phenotype can be reverted by the expression of NUMB, a Notch antagonist [24]. Notch signals possibly increase cell proliferation through activation of its downstream target c-myc. C-myc is a transcription factor, whose deregulated expression has been observed in a large variety of cancers.

The Role of Notch Signaling in Embryonic Stem Cells

Recent studies have demonstrated that Notch signaling is not active in undifferentiated human ESC and not required for the maintenance of pluripotency in human ESC [25]. In this study, Notch signaling was inhibited by culturing cells in the presence of the y-secreatse inhibitor *N*-[*N*-(3, 5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycin *t*-butyl ester (DAPT). Inhibition of Notch signaling did not affect the undifferentiated cells but significantly reduced the proportion of differentiating cells that normally appear within a human ESC culture [25]. Consistent with this model, Notch signaling increased in differentiated cells derived from a colony of hESC. Furthermore, cells differentiated in the presence of constitutive Notch signaling exclusively adopt a neural cell fate [26]. Thus, although Notch does not appear to play a role in maintenance of pluripotency in hESC, it is involved in cell fate decisions and misregulation

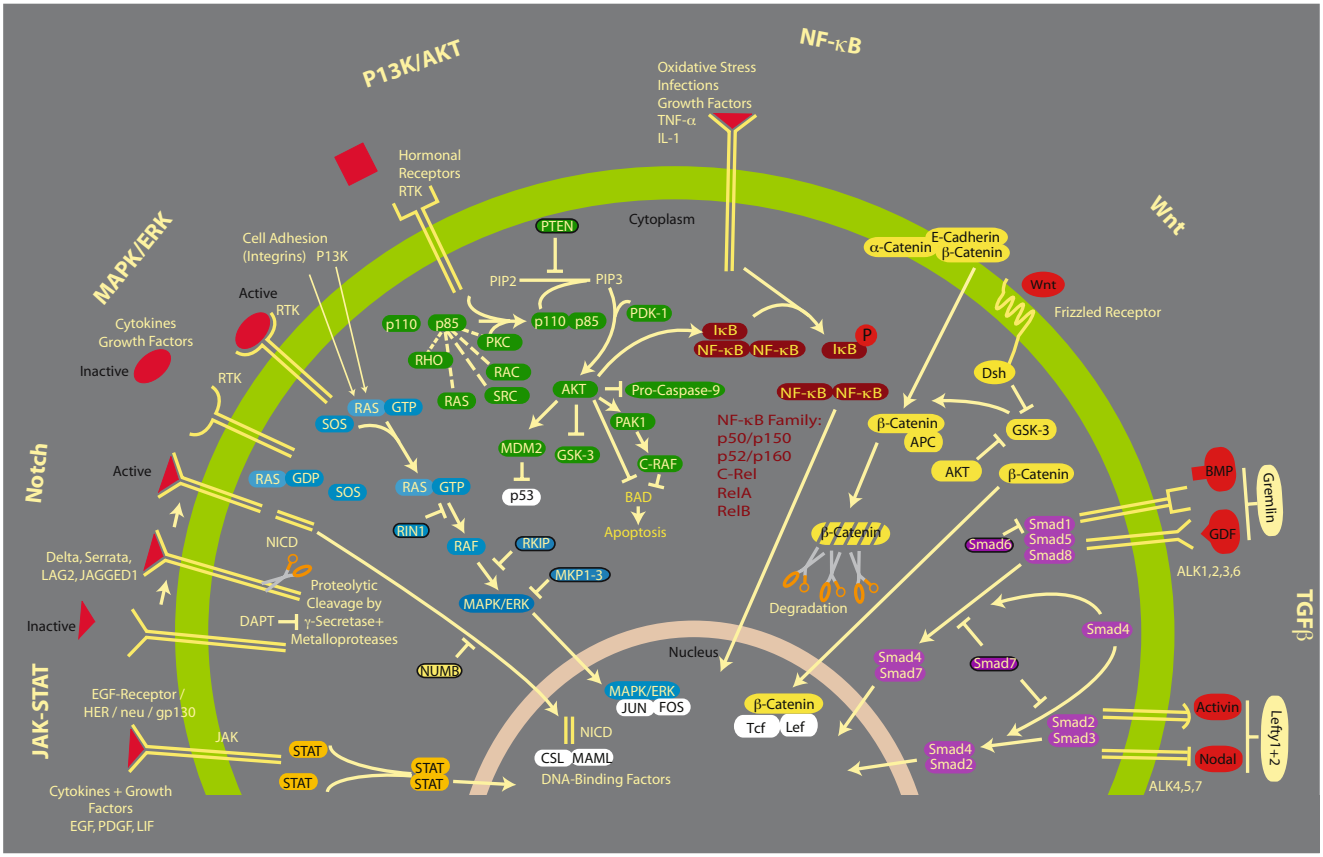

Fig. 1 Signaling networks in cancer and stem cells. Schematic representation of the seven signaling pathways involved in cancer and stem cells: JAK/STAT (*orange*), Notch, MAPK/ERK (*blue*), PI3K/AKT (*green*), NFκB (*brown*), Wnt (*yellow*), TGF-β (*purple*). All ligands are colored in *red* and transcription factors are in *white*. Factors that exert an inhibitory effect onto a particular pathway are framed with a *black line*. Cellular membrane and nuclear membranes are drawn in *green* and *grey,* respectively

of this pathway has been found in a variety of human cancers.

The Map Kinase/Erk Pathway

As we discussed above, growth-promoting signals are relayed to the interior of the cell through a series of different pathways. The RAS-Mitogen Activated Protein Kinase (MAPK) pathway plays an integral part in transducing signals from cytokines and growth factors through Receptor Tyrosine Kinases (RTK) to promote cell adhesion, proliferation, migration and survival [27]. Central to this signaling cascade is RAS, a small membrane-bound GTPase that shuttles between two conformational states: active GTP-bound and inactive GDP-bound. RAS is activated by Son Of Sevenless (SOS), a guanine exchange factor usually found in the cytoplasm of the cell. Upon receptor signaling, SOS shuttles to the cell membrane to catalyze the nucleotide exchange reaction of RAS. Activated GTP-bound RAS activates the serine/threonine kinase RAF that, in turn activates mitogen-activated protein kinases (MAPK) also known as extracellular signal-regulated kinase (ERK). ERK1/2 translocate to the nucleus where they activate the Jun/Fos transcription factors. Numerous factors inhibit the activity of this pathway: RKIP, RAF Kinase Inhibitor Protein obstructs phosphorylation of MAPK; RAS and RAB Interactor 1 (RIN1) competes with RAF for binding of GTP-bound RAS; MKP1–3, MAPK Phosphatases abolish signal transmission by dephosphorylation of MAPK/ERK. Lastly, the MAPK pathway can also be activated by extracellular matrix molecules and changes in focal adhesion, however, most of these signals are relayed through Focal Adhesion Kinase (FAK) and Phosphatidylinositol 3-Kinase (PI3K) which will be discussed later.

MAPK/ERK Pathway in Cancer

Because of its central role in cell proliferation, it is not surprising that the SOS-Ras-Raf-MAPK signaling cascade is deregulated in a broad spectrum of human tumors. Most of these mutations occur in RAS and RAF and result in constitutive pathway activation and the adoption of a

hyperproliferative state. RAS mutations are found in ~45% of colon cancers and ~90% of pancreatic cancers [27]. RAF mutations, in turn, are found in roughly two thirds of all melanoma [28]. Consequently this pathway is a formidable target for therapeutic intervention and has received tremendous attention.

MAPK/ ERK Pathway in Stem Cells

Armstrong et al. demonstrated by microarray analysis and western blotting that components of the SOS-RAS-RAF-MAPK pathway, such as RASAL2 and SOS1 are downregulated more than four-fold during differentiation of hESC [29]. Other components, including MAP4K1, MAP2K6, RAF, KRAS, NRAS and BRAF were downregulated less than four-fold [29]. Consistent with these results, immunohistochemical analysis revealed that phosphorylated c-RAF and SOS1 were present at the cell membrane and within the cytoplasm and nucleus. Undifferentiated ESC require the growth promoting factor FGF, which signals through the RAS/ERK/MAPK pathway. Indeed, inhibition of MAPK/ERK pathway with U0126, in the presence of FGF led to massive cell death and differentiation [29]. Non-physiological levels of FGF sustain pluripotency of hESC in the absence of a feeder layer. Taken together, these results suggest that MAPK/ERK signaling is active in undifferentiated hESC and downregulated upon differentiation.

PI3K/AKT Pathway

Similar to the MAPK/ERK pathway, the phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway responds to a variety of extra- and intracellular signals, relayed through hormonal receptors, transmembrane tyrosine kinase-linked receptors (RTK) and intracellular factors and regulates cellular proliferation, cell death and cytoskeletal rearrangements [30]. Class I PI3K are heterodimers, consisting of an adaptor / regulatory p85 subunit and a p110 catalytic kinase subunit. Activation of classI PI3K occurs through interaction of the p85 subunit with various activating proteins such as protein kinase C, RHO, RAC, mutated RAS, SRC and leads to activation of the p110 catalytic subunit. Upon activation, PI3K phosphorylates phosphatidylinositol-4, 5-biphosphate (PIP2) at a 3-position, converting it to phosphatidylinositol-3,4,5-biphosphate (PIP3). PI3K/AKT signaling is counteracted by the Phosphatase and TENsin homologue (PTEN) (and SHIP1, SHIP2), which dephosphorylates PIP3–PIP2.

One major downstream mediator of PIP3 is AKT. The AKT protein kinase binds PIP3 with high affinity through its Pleckstrin Homology (PH) domain. Activated AKT is localized at the cell membrane where it interacts with and is phosphorylated by Phophoinositide Kinase 1 (PDK1). As we will discuss below, activated AKT regulates a number of downstream targets, implicated in a variety of human diseases.

PI3K/AKT Signaling in Cancer

Activated PI3K/AKT signaling has a variety of downstream effectors that mediate cellular energy metabolism, cell proliferation and survival. There is significant cross-talk between the PI3K/AKT and other pathways, such as the apoptosis pathways and the nuclear factor-kappa-B (NF-κB) and possibly the Wnt pathway that we will discuss below. With respect to apoptosis, activated AKT directly and indirectly (through PAK1 and c-RAF) inhibits apoptosis mediators BAD and pro-caspase 9 [31]. On a transcriptional level, AKT upregulates transcription of the NF-κB inhibitor I-κB. Furthermore, PDK1 phosphorylates a negative regulator of NF-κB signaling, IKKβ and targets it for degradation. AKT directly activates MDM2, a negative regulator of p53. p53 is responsible for DNA damage surveillance and in response, initiates cell cycle arrest and DNA repair. Interestingly, AKT also inhibits Glycogen Synthase Kinase-3 (GSK-3), a negative regulator of Wnt signaling. Deregulated PI3K/AKT signaling has been observed in various cancers. Mutations in the PI3K/AKT pathway inhibitor and tumor suppressor PTEN has been found in glioblastomas, lung carcinomas and melanomas whereas AKT overexpression or overactivation has been found in breast, ovarian, Thyroid and a variety of other cancers [31]. In conclusion, the PI3K/AKT pathway is constitutively active in numerous human cancers. Activation of this pathway can promote cell survival and proliferation. However, PI3K/AKT signaling further stimulates proliferation by activation of the NF-κB and Wnt signaling pathways (discussed below).

PI3K/AKT Signaling in Embryonic Stem Cells

The PI3K signaling pathway has been shown to be important to maintain pluripotency in mouse ESC [32]. Expression of a dominant-negative allele of PI3K or blockage of the pathway by a PI3K inhibitor LY294002 initiated cellular differentiation [33]. Microarray analysis of hESC indicated that components of the PI3K/AKT pathway are upregulated in undifferentiated ESC [29]. To see whether PI3K signaling is necessary to maintain pluripotency in hESC, colonies were treated with PI3K inhibitor LY294002. Inhibition of PI3K signaling was confirmed by checking the phosphorylation status of AKT, c-RAF and GSK-3; all of which showed decreased levels of phosphorylation. Markers of pluripotency such as OCT4, NANOG and SOX2 decreased, indicating that the cells lost their

pluripotent capabilities and initiated differentiation. However, considering that the Wnt and NF-κB signaling pathways are activated downstream of the PI3K/AKT pathway, loss of pluripotency could be attributed to insufficient activation of these pathways.

The NF-κB Pathway

The nuclear factor-kappa-B (NF-κB) pathway regulates genes involved in key cellular processes such as proliferation, stress response, innate immunity and inflammation [12, 34]. In vertebrates, the NF-κB transcription factor family consists of p50/p105, p52/p100, c-Rel, RelA and RelB that regulate transcriptional expression of hundreds of target genes. P105 and p100 are proteolytically processed to give rise to p50 and p52, respectively. In contrast c-Rel, RelA and RelB contain a C-terminal transactivation domain and are not processed. c-Rel, RelA, RelB, p50 and p52 can form homo- and heterodimers, shuttle to the nucleus where they bind DNA regulatory κB sites. In the absence of signaling, NF-κB dimers are located in the cytoplasm and inactivated by their interaction with I-κB inhibitory proteins. NF-κB signaling is activated by a variety of extracellular factors such as the tumor necrosis factor-α (TNF-α), interleukin-1, growth factors, bacterial or viral infections, oxidative stress and pharmaceutical compounds. In response to such stimuli, I-κB is rapidly phosphorylated on serine 32 and 36 by the I-κB kinase (IKK). Phosphorylated I-κB is ubiquitinated by the E3 ubiquitin ligase complex and targeted for degradation by the 26S proteasome. The liberated NF-κB dimers can then translocate to the nucleus and activate transcription of target genes.

NF-κB Signaling in Human Cancer

Mutations and miss-regulation of NF-κB signaling has been involved in a variety of cancers, for example human B-cell malignancies. The human REL gene, encoding one of the five NF-κB transcription factors is amplified in ~50% of Hodgkin's lymphoma, ~10–20% of non-Hodgkin's B-cell lymphomas and ~40% natural killer T-cells lymphomas. It has been suggested that amplification of the REL gene and overexpression of the protein outcompetes the inhibitory effects of I-κB in the cytoplasm, leading to constitutive transcription of NF-κB target genes and increased mature B-cell proliferation and survival. Consistent with this model, overexpression of human REL is sufficient to transform and immortalize primary chicken lymphoid cells in culture, whereas diminished levels of REL inhibit B-cell proliferation [35]. Immunohistochemical analysis of patient-derived lymphoma samples with REL amplifications has confirmed nuclear REL expression in several cases [36].

NF-κB Signaling in Embryonic Stem Cells

Very little is known about the role of NF-κB signaling in human and mouse ESC. However, recent studies assessed their expression in several lines of undifferentiated and differentiated ESC using microarray expression profiling, western blotting, flow cytometry and antibody arrays [29]. Consistently, components of the NF-κB signaling pathway were enriched in undifferentiated hESC, and downregulated during differentiation. Immunohistochemistry of RelA showed that it is only present in the nucleus of undifferentiated hESC, indicating active NF-κB signaling. To test whether NF-κB signaling was necessary to maintain pluripotency, sodium pyrrolidinethiocarbamate (PTDC), a specific NF-κB inhibitor, was added to the cells. PTDC prevents RelA (p65) entry into the nucleus. Inhibition of NF-κB signaling by PTDC caused massive cell differentiation within ESC colonies, as well as significant cell death, demonstrating that NF-κB is essential to maintain ESC pluripotency [29].

The Wnt Pathway

The Wnt signaling pathway is among the evolutionary most conserved pathways, implicated in a variety of cellular, embryological and physiological activities from *C. elegans* to humans. The Wnt pathway has three branches: the Ca++, the planar polarity, and the canonical branch. Among these three, the canonical branch of the pathway has been implicated in tumorigenesis as well as the maintenance of the state of pluripotency ("stemness") in stem cells. The canonical branch of the Wnt pathway has been shown to be causal to a variety of tumors, including colon cancer and breast cancer. The same branch of the pathway has been shown to be necessary and sufficient to maintain the state of pluripotency, in both embryonic stem cells, as well as adult stem cells of at least two origins: the hematopoeitic cells of the bone marrow and epidermal stem cells.

The Wnt pathway consists of more than 30 extracellular Wnt-ligands, which interact with receptors of the frizzled family [37]. Engagement of Wnt ligands with the receptor activates a protein called Dishelved (Dsh). Dsh inhibits the Glycogen-Activated Kinase-3 (GSK-3), which, in the absence of Wnt signaling phosphorylates and targets the β-catenin-Adenomatous Polyposis Coli (APC) complex for ubiquitination and proteolytic degradation. Upon Wnt signaling, β-catenin is stabilized, accumulates in the cytoplasm and translocates to the nucleus, where it interacts with DNA-binding proteins of the T-cell Factor / Lymphocyte Enhancer binding Factor (Tcf/Lef) family. In the presence of β-catenin, Tcf/Lef act as transcriptional

activators of proliferation stimulating genes such as c-myc and cyclin D1 [38].

Wnt Signaling in Cancer

Homeostatic replacement occurs at various frequencies in many epithelia of the human body and Wnt signaling plays a key role in this process. For example, intestinal epithelia are replaced every week. Other epithelia, such as the interfollicular epidermis are replaced every month, whereas the lung epithelium takes up to 6 months to self-renew [39]. The colon can be imagined as a two-layered sheet of cells. The top layer is replenished by proliferative stem and precursor cells, which reside in the bottom layer. The stem cells constitutively proliferate and produce progenitor cells, which subsequently differentiate into non-dividing epithelial cells on the top. Wnt-signaling is active in the cycling stem cells that reside in the bottom layer and turned off upon differentiation and migration to the top layer. In a majority of colon cancers (90%), APC, which under normal circumstances targets β-catenin for degradation, is mutated giving rise to β-catenin stabilization and initiation of the Tcf/Lef transcriptional program [40]. Thus, APC deficient progenitor cells keep replicating, persist within the tissue, and run the risk of acquiring further mutations that turn them malignant [41]. Similarly, Wnt signaling activating mutations in APC or β-catenin are frequently found in small intestinal adenocarcinomas (48%) and gastric polyps (64%) [40]. Aberrant Wnt-signaling is also found in chronic and acute myeloid leukemia. Activation of β-catenin increases the self-renewal capacity of a subpopulation of hematopoetic progenitor cells and it has been hypothesized that during this abnormally extended period of self-renewal, granulocyte-macrophage progenitors (GMPs) acquire leukemia characteristic translocations such as BCR-ABL [41, 42].

Wnt Signaling in Stem Cell Self-renewal

Wnt-signaling has also been shown to play a crucial role in sustaining self-renewal in both embryonic and adult stem cells in mammals [19]. In the context of embryonic stem cells, it has been shown that the pharmacological inhibitor 6-bromoindirubin-3′oxime (BIO) blocks GSK-3 in both human and mouse ESC [19]. GSK-3 negatively regulates Wnt signaling by phosphorylating the amino terminus of β-catenin. Phosphorylated β-catenin is ubiquitinated and targeted for proteolytic degradation. In the presence of BIO, β-catenin accumulates and translocates to the nucleus where it engages with Tcf/Lef and activates transcription of genes involved in self-renewal. As judged by expression of pluripotency markers such as Oct3/4, Nanog and Rex1, BIO maintained the undifferentiated state of human ES cells for several passages. BIO treated ESC retained the differentiation potential and gave rise to embryoid bodies or teratomas containing derivatives of all three germ layers. Upon withdrawal of BIO or after extended passaging, BIO treated cells differentiated [19]. Further optimization will be needed to enable the cells to remain in the undifferentiated state for extended periods of time. Nevertheless, these experiments demonstrated the potential of small molecules such as BIO in optimizing ESC culture conditions. Interestingly, inhibition of GSK-3 and subsequent activation of Wnt-signaling also led to activation of the SMAD2/3 branch of the TGF-β pathway (discussed below), demonstrating that there is crosstalk between these two pathways [43].

In adult stem cells the canonical branch of the Wnt pathway has been shown to be necessary for the maintenance of pluripotency in skin and hematopoietic stem cells. In the skin epithelial tissue homeostasis is maintained by adult stem cells. Epithelial cells of the skin form a tight layer through the formation of tight junctions, adherence junctions and desmosomes. In these differentiated cells, the vast majority of β-catenin is located at the cell membrane where it interacts with E-cadherin and alpha-catenin at adherence junctions. In adult stem cells of the skin as well as in hair follicle stem cells, B-catenin is enriched in the cytoplasm and nucleus where it functions in self-renewal and lineage determination. Deletion of β-catenin in mice impairs hair follicle morphogenesis and loss of the follicle stem cell niche [39]. Furthermore, stabilization of β-catenin drives hair follicle stem cells into proliferation and regeneration of hair follicles, and has been linked to pilomatricomas, tumors of the hair shaft cells. In conclusion, Wnt-signaling is necessary to maintain pluripotency in ESC and mutations in the Wnt-pathway have been linked to a variety of human epithelial carcinomas.

The TGF-β Pathway

This pathway was first discovered in tumors as an anti-proliferative signal, critical for maintaining tissue homeostasis by keeping cell proliferation in check. Growth inhibitory signals exist as soluble factors, within the extracellular matrix or the surface of neighboring cells, and send a cell into a reversible growth arrest called quiescence. The Transforming Growth Factor-β (TGF-β) pathway plays a key role in executing this task. Just like the Wnt pathway, the TGF-β pathway has three main branches: the SMAD1/5/8, the SMAD2/3 and the TAB/TAK branch. With 42 ligands in the human genome the TGF-β pathway, which is also evolutionarily conserved, represents one of the most complicated pathways in metazoans. The activation of the pathway is mediated by TGF-β ligands binding to the extracellular domain of Type I and Type II TGF-β receptors. By direct serine phosphorylation the signal is

transmitted to latent cytoplasmic transcription factors, the SMADs. The TGF-β signaling pathway consists of two main branches: a) Bone Morphogenic Protein (BMP) and Growth Differentiating Factor (GDF) ligands bind Type I receptors ALK1, ALK2, ALK 3 and ALK6, leading to phosphorylation and activation of SMAD1 and 5. Activin and Nodal ligands bind ALK4, ALK5 and ALK7 receptors and trigger phosphorylation and activation of SMAD2 and SMAD3. Phosphorylated SMADs form a higher-order complex with SMAD4, translocate to the nucleus and regulate transcription of a broad range of genes. TGF-β signaling can be inhibited in a cell non-autonomous and cell-autonomous fashion. Cell autonomous inhibitors are SMAD6 and SMAD7. SMAD6 negatively regulates TGF-β signaling by inhibition of SMAD1/5 whereas SMAD7 inhibits both TGF-β signaling branches by blocking the interaction of SMADs with SMAD4 [12]. Cell non-autonomous TGF-β inhibitors are Lefty-1 and Lefty-2, Cerberus, Follistatin, Chordin and drm/gremlin. Lefty 1 + 2 inhibit TGF-β signaling through SMAD2/3 by binding to Nodal or by preventing the assembly of the Nodal/Activin receptor complex. In human ESC, Gremlin inhibits BMPs and GDFs and blocks TGF-β signaling through the ALK2/3/6—SMAD1/5/8 branch of the pathway.

TGF-β Signaling in Cancer

Mutations or downregulation of TGF-β receptors, inactivation of SMAD4 or $p15^{INK4B}$ can be found in a variety of cancers. In particular SMAD4 inactivation occurs in ~53% of human pancreatic ductal adenocarcinomas (PDAC) [44, 45]. Furthermore, BMP2 is dramatically overexpressed in 98% of lung carcinomas [46, 47]. Co-injection of recombinant BMP2 and lung cancer cells into nude mice led to increased tumor growth in the lungs. Furthermore, BMP2 induced phosphorylation and nuclear accumulation of SMAD1/5. In contrast, expression of BMP-antagonist Noggin reduced tumor growth [46]. BMP2 did not promote growth of subcutaneous tumors and it has also been shown to inhibit growth of some cells lines in vitro [47]. Thus, BMP2's growth promoting activities clearly depend on other environmental factors.

TGF-β signaling can also enhance malignancy of epithelial tumors by stimulating metastasis; a complex process during which a cell disintegrates from the original tumor, acquires increased motility and invasiveness to enter the circulatory system and colonizes a new niche within the body. In many ways, metastasis resembles the highly coordinated cell movements that occur during embryonic development. During gastrulation in higher vertebrates, cells of the anterior primitive streak disjoin from their neighbors and ingress into the interior of the embryo. During this process, the phenotype of these cells changes from epithelial to mesenchymal, termed the epithelial-to-mesenchymal transition (EMT). EMT during metastasis causes cellular adhesion molecules, such as E-cadherins and cytokeratins, to be replaced by mesenchymal proteins N-cadherin and vimentin [48]. Strikingly, pathways and genes involved in EMT during development such as TGF-β, Goosecoid or TWIST, are re-expressed during tumorigenesis and can initiate metastasis [49–51]. In conclusion, the TGF-β signaling pathway can act as accelerator or break to regulate cell proliferation and plays a pivotal role in cancer metastasis [7].

TGF-β Signaling and Self-renewal in Stem Cells

It has long been appreciated that the TGF-β pathway plays a crucial role during embryonic development. Its components are well evolutionarily conserved and play a pivotal role in the determination of cell fate, such as neural induction and mesoderm specification in Xenopus, as well as mesoderm and primitive streak formation in mouse. Several lines of evidence suggest that TGF-β signaling is also involved in sustaining the undifferentiated state in hESC. First, transcripts of TGF-β ligands GDF-3, BMP2 and TGF-β1, as well as the membrane-bound components, Lefty A, Lefty B, Cripto, Cerberus and TMEFF are enriched in undifferentiated hESC [52]. Secondly, SMAD2 and SMAD3 are both (c-terminally) phosphorylated and localized in the nucleus, indicating that the Nodal / Activin branch of TGF-β signaling is active [53]. Upon differentiation, SMAD2/3 phosphorylation levels decreased [43]. Furthermore, inhibition of SMAD2/3 phosphorylation by a chemical kinase inhibitor SB-431542 initiated differentiation. Taken together, these results suggest that the Activin / Nodal branch of TGF-β signaling is necessary to sustain pluripotency. In contrast the BMP branch of TGF-β signaling appears to play the opposite role. In undifferentiated cells, SMAD1/5 phosphorylation levels are hardly detectable but increase upon differentiation [43, 53]. Consistent with these findings, treatment of ESC with BMP4 initiated differentiation to trophoblast [54]. These results are in agreement with previous studies that deleted several components of the TGF-β pathway and investigated its consequences in the context of early mouse development. Although cell fate specification was impaired or absent, none of the mutant embryos was impaired in establishment or maintenance of the pre-implantation stem cell compartment [55].

More recently, a group explored the BMP4 ligand to target tumor-initiating stem cell-like precursors in human glioblastomas [56]. Treatment of human glioblastomas with BMP4 activated the BMP branch of TGF-β signaling, initiated neural differentiation and blocked tumor growth in a mouse model. Although these experiments raised a

Table 1 Status of signaling pathways in cancer and undifferentiated hESC

Pathway	Cancer/tumor types	hESC (undifferentiated)
JAK/STAT	↑STAT3 50% lung 95% head and neck	inactive
Notch	↑Notch 55–60% T-cell acute lymphoblastic leukemia (T-ALL)	inactive
MAPK/ERK	↑RAS in 45% colon 90% pancreatic cancers ↑RAF ~66% melanoma	active
PI3K/AKT	↑AKT in breast, ovarian & Thyroid ↓PTEN in glioblastomas, melanoma and lung carcinoma	active
NF-κB	↑ REL 50% of Hodgkin's Lymphoma; 40% NTK lymphomas	active
Wnt	↑β-catenin in 48% of small intestinal adenocarcinomas ↑β-catenin in 64% of gastric polyps ↓APC in 90% of colon cancers ↓APC in 76% of gastric adenomas	active
TGF-β	↓SMAD4 53% pancreatic carcinomas ↑BMP2 in 98% of Lung carcinomas	only Activin/Nodal branch is active

promising possibility that BMPs could be used as a therapeutic intervention for human glioblastomas, they did not address whether BMP treatment has any secondary effects on non-transformed "normal" neural stem cells [56].

In conclusion, extensive studies in Xenopus and mouse established the TGF-β signaling pathway as a major regulator during embryonic development. More recent studies have demonstrated the importance of this pathway in maintaining pluripotency and self-renewal in human embryonic stem cells.

Conclusions and Perspectives

In this review we have addressed our current state of understanding of signaling pathways involved in pluripotency of human embryonic stem cells, and its comparison with signaling pathways involved in cancer (Table 1). Through the collective effort of many groups, the signaling networks required for the maintenance of "stemness" are being unraveled, and the amazing correlation of these pathways to cancer pathways, as well as common cellular properties between embryonic stem cells and cancer cells, can no longer be dismissed as coincidence. The resolution of these parallels will inevitably impact both clinical, as well as basic sciences. The availability of human embryonic stem cells opens a window to the study of human embryology and provides the unprecedented opportunity to ascertain the amount of the molecular information, obtained from developmental studies of model systems, such as frog, chick and mouse, that is relevant to human development. Finally, since hESC could constitute a renewable source of a large variety of differentiated cells, they could be employed to replace diseased or damaged tissue by cellular transplantation. Therefore, the understanding of molecular mechanisms and cracking the code of the signaling networks that orchestrates stemness, as well as a global and basic molecular knowledge of their pluripotency, is an obligatory step towards the design of rational clinical treatments.

Acknowledgments We are grateful to the members of the Brivanlou lab, in particular Scott Noggle, Ariel Levine and Blaine Cooper for helpful discussions and assistance during the preparation of this manuscript. Andre Hoelz is acknowledged for inspiring discussions. This work was supported by NIH grant GM073370 to A.H. B. and a Zuckerman family postdoctoral fellowship to O.D.

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