Neural is Fundamental: Neural Stemness as the Ground State of Cell Tumorigenicity and Differentiation Potential

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Abstract
Tumorigenic cells are similar to neural stem cells or embryonic neural cells in regulatory networks, tumorigenicity and pluripotent differentiation potential. By integrating the evidence from developmental biology, tumor biology and evolution, I will make a detailed discussion on the observations and propose that neural stemness underlies two coupled cell properties, tumorigenicity and pluripotent differentiation potential. Neural stemness property of tumorigenic cells can hopefully integrate different observations/concepts underlying tumorigenesis. Neural stem cells and tumorigenic cells share regulatory networks; both exhibit neural stemness, tumorigenicity and pluripotent differentiation potential; both depend on expression or activation of ancestral genes; both rely primarily on aerobic glycolytic metabolism; both can differentiate into various cells/tissues that are derived from three germ layers, leading to tumor formation resembling severely disorganized or more degenerated process of embryonic tissue differentiation; both are enriched in long genes with more splice variants that provide more plastic scaffolds for cell differentiation, etc. Neural regulatory networks, which include higher levels of basic machineries of cell physiological functions and developmental programs, work concertedly to define a basic state with fast cell cycle and proliferation. This is predestined by the evolutionary advantage of neural state, the ground or initial state for multicellularity with adaptation to an ancient environment. Tumorigenesis might represent a process of restoration of neural ground state, thereby restoring a state with fast proliferation and pluripotent differentiation potential in somatic cells. Tumorigenesis and pluripotent differentiation potential might be better understood from understanding neural stemness, and cancer therapy should benefit more from targeting neural stemness.

Keywords Neural stemness · Neural ground state · Neural default model · Differentiation potential · Tumorigenic cell · Tumorigenicity · Unicellularity · Multicellularity · Evo-devo · Cancer stem cell

Introduction
Cancer has been a disease that is difficult for research and therapy since its earliest description in an ancient Egyptian textbook about 5,000 years ago [1]. The difficulty resides in the extreme complexity of cancer, as reflected by enormous inter- and intra-tumor heterogeneity, including genetic heterogeneity in most tumors caused by genetic changes and phenotypic heterogeneity in a tumor. Heterogeneity can also be the consequences of epigenetic and microenvironment changes [2–6]. There have been many hypotheses and concepts trying to explain the phenomenon of cancer initiation and progression [7, 8]. They can explain tumorigenesis in some cases, but meet serious challenges in others [8]. In this review, I will discuss my recent findings, in combination with other studies and clinical evidence, about a general rule of tumorigenesis. This grave biological phenomenon might have been suggesting a general principle of cell biology, which means that evolutionarily pre-determined state of neural stemness is the ground state for cell tumorigenicity and pluripotent differentiation potential.

The ‘Neural Default Model’ of Embryonic Pluripotent Cells
The link between neural stemness, cell tumorigenicity and pluripotent differentiation potential can be traced to the ‘neural default model’ of embryonic pluripotent cells...
in developmental biology. At the beginning of embryogenesis, a fertilized egg cleaves to generate blastomeres that further differentiate into three primary germ layers, ectoderm, endoderm and mesoderm. Ectoderm is the germ layer giving rise to both epidermis and nervous system, and other tissues/ organs are derived from either endoderm or mesoderm. How the neural tissue in an early embryo is induced to form had been a major topic of research in developmental biology. The pioneering work in 1924 was that the dorsal blastopore lip, or the Spemann organizer, of an early newt gastrula embryo was able to induce neural plate, the precursor tissue for central nervous system, in ectoderm, while the dorsal lip itself developed into mesodermal notochord [9]. However, the nature of the neural inducing activity in the organizer had not been understood for six decades until the emergence of some critical initial findings at the end of 1980 s. Isolated blastula ectoderm differentiates into epidermis when cultured in neutral saline in vitro. Surprisingly, these ectodermal cells differentiate exclusively into neural tissues instead when they are disaggregated first for a few hours and then re-aggregated again in culture [10–12]. This means that absence, but not presence, of an extracellular signal is prerequisite for neural fate decision, and neural fate might be the default fate of ectoderm. Subsequent mechanistic studies elucidated that the organizer is a wealth of secreted factors antagonizing BMP4, a TGFbeta ligand that transduces epidermis-inducing and anti-neural signaling in ectoderm [9, 13–15]. Contrary to the initial aim of search for neural inducers, these studies reveal a non-obvious principle that neural fate is achieved by default and epidermal fate is induced during ectodermal cell fate decision, i.e., the ‘neural default model’ of ectoderm [9, 16]. Besides neural and epidermal differentiation, blastula ectoderm can also be induced into mesodermal and endodermal tissues in response to inducers, such as Activin. The pluripotent differentiation potential of blastula ectodermal cells resembles that of mammalian embryonic stem cells (ESCs), which are derived from inner cell mass of mammalian blastocyst embryos and are capable of differentiation into different cell types of all three germ layers. The neural default fate is also manifested by ESCs. When ESCs are cultured in defined serum-free medium instead of regular medium that contains high concentration of fetal bovine serum, the cells adopt a neural fate and turn into primitive neural stem cells (primNSCs) [17–19], i.e., NSCs at the earliest stage of neural differentiation from embryonic ectoderm. BMP4 signaling inhibits neural differentiation in amphibian ectodermal cells, which adopt a neural fate in the absence of BMP4. Likewise, it is required for maintenance of ESC pluripotency because ESCs without BMP signaling will also adopt a neural fate [20, 21]. The neural default state of embryonic pluripotent cells provides the mechanism for cell fate decision between neural and non-neural cells at the beginning stage of differentiation during embryogenesis. If considering that embryonic pluripotent cells can be induced to differentiate into various types of non-neural somatic cells by different lineage or cell type specific factors, it can be deduced that the ‘neural default state’ can be extended to and applied for somatic cells. Taken together with the tumorigenic property of embryonic pluripotent cells [22], our studies and studies from other groups provide the evidence that neural state represents the ground state of cell differentiation and tumorigenicity.

The Link Between Neural Stemness and Cancer Cells

Downregulation/silencing of pro-differentiation genes or/ and tissue-specific genes is a common feature of tumorigenesis, leading to a dedifferentiation effect and gain of stemness in cancer cells [4, 23, 24]. It is expected that re-differentiation will cause a reduction or loss in cancer cell malignancy. In an attempt to drive terminal differentiation of cancer cells, we found that blocking epigenetic modification factors, HDAC1/3, EZH2, LSD1 and DNMT1, could lead to postmitotic neuron-like differentiation in cell lines of different cancers, accompanied with dramatic reduction in malignant features and tumorigenicity in these cells [23, 25]. These proteins play extensively promoting roles and expression of their genes is upregulated or activated during initiation and progression of different cancers. Neuronal differentiation is the key property of neural stem/progenitor cells (NSCs/NPCs). The neuronal phenotype suggests that different cancer cells may have the property of NSCs/ NPCs. In agreement, neuronal differentiation phenotype in cancers has been reported in many studies [26–31]. Compatible with the property of neural stemness is the specific expression of genes of these proteins in embryonic neural cells. Comprehensive analyses on more than 3,000 cancer related genes demonstrated that most genes promoting tumorigenesis and/or being upregulated/activated during tumorigenesis are enriched in embryonic neural tissues or NSCs. In contrast, genes suppressing tumorigenesis and/ or being downregulated/silenced during tumorigenesis are generally not expressed in embryonic neural cells [23]. This means that cancer cells and embryonic neural cells share numerous regulatory signals, which confer the property of NSCs/NPCs to cancer cells. The genes that regulate different malignant features, like mobility, proliferation, chemoresistance, stemness, dysregulated epigenetics and metabolism, etc., are all corresponding with neural specific or neural enriched genes. Therefore, I proposed that tumorigenesis might represent a process of gradual loss of original cell identity and gain of the property of NSCs/NPCs [24]. Regulation of tumorigenesis includes thousands of cancer
related genes and numerous intertwined signaling pathways. In fact, nearly all aspects of biological research have been reported to be involved in tumorigenesis. This makes tumorigenesis an incomprehensible biological phenomenon if no links between different regulatory signals are established. Due to that tumorigenesis is characterized by upregulation/activation of cancer-promoting genes, which are enriched in embryonic neural cells or NSCs, and downregulation of tissue-specific genes, the complexity of tumorigenesis might be reduced as a process of gain of the property of a particular cell type, the neural stemness.

There are quite some scattered pieces of evidence in clinical and basic studies showing the association between tumorigenesis and neural cells, as reviewed in Ref. 24 [24]. It was reported that NPCs promote cancer [32]. A piece of more direct evidence showed that loss of a transcriptional repressor causes transition of intestinal stem cells in *Drosophila* into NSC-like state and drives neuroendocrine tumor formation [33]. Although cancer cells and NSCs/NPCs have similar neuronal differentiation potential (i.e., neural stemness), and share numerous regulatory signals, the key question is how neural stemness is related with cell tumorigenicity.

**Neural Stemness as the Source of Cell Tumorigenicity**

Among embryonic and adult cell types, ESCs and their artificial equivalent, the induced pluripotent stem cells (iPSCs), have tumorigenic potential [22]. When transplanted into immunodeficient mice, these cells form teratomas that contain tissue types derived from all three germ layers. Teratoma formation is also a standard assay for investigating pluripotent differentiation potential of ESCs or iPSCs [34]. NSCs/NPCs are considered as a type of somatic stem cells, which have been thought as non-tumorigenic [35]. However, occasional studies revealed the opposite evidence. PrimNSCs derived from ESCs or iPSCs show tumorigenicity in immunodeficient mice [36, 37]. The derived primNSCs were even purified to exclude the tumorigenic effect of some remaining cells that did not change fully into primNSCs [36]. The mechanisms for this effect are unknown. The most convenient explanation is due to the leftover of incompletely differentiated ESCs or the expression of MYC oncoprotein in iPSCs. Another report complicates this assumption, because tumor formation derived from transplanted human fetal NSCs in a patient was observed in a clinical practice [38]. If we consider the neural default state and tumorigenicity of ESCs, tumorigenicity of NSCs is not irrational. As a matter of fact, tumorigenicity is an intrinsic property of NSCs/NPCs [39]. First, tumorigenicity was demonstrated for primNSCs that were derived from ESCs. This should not be due to the remnant of incompletely differentiated ESCs because the former show stronger tumorigenicity than the latter. Actually, TGFbeta signaling is required for inhibition of neural differentiation and promotes non-neural differentiation of ESCs [17, 20, 40]. It suppresses tumor formation while promotes metastasis, immune regulation, etc., of cancer cells [41, 42]. It is rather logical that tumorigenic cells are stronger in tumorigenesis in the absence of TGFbeta signaling. PrimNSC-derived tumors are composed of tissues of all germ layers, including immature neuroectodermal cells, differentiated neuronal cells, or keratinized structures from ectoderm, and full spectrum of tissues from mesoderm and endoderm [39]. These tumors are very similar to teratoma formed by ESCs, suggesting that primNSCs, representing initial neuroectodermal cells, are pluripotent in differentiation potential. Second, tumorigenicity was demonstrated for a NSC cell line derived from E9 mouse embryos [39]. Subcutaneous transplantation of the cells caused formation of tumors, and tail vein injection of the cells led to formation of tumors in various tissues or regions of mouse body. The tumors contain also different tissues of three germ layers, but with less abundance of tissue types than in primNSC-tumors. Third, tumorigenicity was demonstrated for NPCs that were isolated from cortices of E13.5 mouse embryos. Different tissue types of neural and non-neural lineages can also be observed in these tumors. Moreover, NSCs/NPCs contribute to chimera formation in chick and mouse embryos [17, 43]. These analyses suggest that NSCs/NPCs have both tumorigenic and pluripotent differentiation potential.

Many types of adult tissue stem/progenitor cells have been identified. However, there seem to be no evidence for that other tissue stem/progenitor cells have tumorigenic potential. Mesenchymal stem cells (MSCs) have been extensively investigated for their roles during tumorigenesis. They exhibit seemingly both a promoting and an inhibitory effect on tumor progression [44]. However, the cells themselves exhibit no tumorigenicity [39, 45–47]. Hematopoietic stem cell transplantation has been widely used as a treatment for hematological cancers and other diseases. In our study, we also observed that embryonic fibroblasts and myoblasts are not tumorigenic [39]. When either the muscle differentiation factor Myod1 was lost in myoblasts or a transcriptional repressor was lost in intestinal stem cells, the resulting cells gained neural stemness and tumorigenicity [33, 39]. In contrast, decrease in neural stemness via differentiation into neuron- or muscle-like cells, tumorigenicity is reduced or lost [23, 39]. These lines of evidence from both NSCs/NPCs and genetically manipulated somatic cells indicate that the property of neural stemness is the source of tumorigenicity.

Both cancer cells and NSCs/NPCs have the neuronal differentiation potential and share similar regulatory networks. Cancer cells behave like NSCs/NPCs in other aspects, such as formation of neurosphere-like structures in defined
NSC serum-free medium. These structures express neural stemness markers, resembling neurospheres of NSCs/NPCs [39]. The ability in neurosphere formation is a reflection of neural stemness and tumorigenicity of cancer cells. Cells without neurosphere formation could mean that they have very weak tumorigenicity, or they are not sufficient to initiate tumor. Weak tumorigenicity could be detected using more sensitive approaches. For example, the osteosarcoma cell U-2OS does not form neurospheres and displays no tumorigenicity in nude mice. It is very weakly tumorigenic in the most severely immunodeficient NOD/SCID IL2R-gamma-0 (NSG) mouse [48]. Non-cancer cells, such as MEFs, myoblasts and MSCs, do not form neurospheres and are not tumorigenic. Tissue stem cells also form spherical structures in serum-free culture, though, the media are different in composition. Specific growth factors or even a specific culture condition is required for a particular type of tissue stem cells. For example, MSCs form spheres in an appropriate serum-free medium [49], but do not in the serum-free medium for neurosphere formation [39]. More importantly, xenograft tumors formed by transplanted NSCs/NPCs or cancer cells show differentiation of tissues or cell types from different lineages, but not the differentiation hierarchy of a type of tissue stem cells along a particular lineage. Neurosphere formation is an indication of neural stemness and tumorigenicity. In line with the experimental observation of pluripotent differentiation potential of NSCs/NPCs [17, 39, 43], bioinformatic analysis also revealed that neural genes are most closely associated with neural development, embryonic development and cancer, whereas non-neural genes are not [39].

The link between neural stemness and cell tumorigenicity has been implied by many lineage-tracing studies. For instances, CD133 (or PROM1) identifies human colon cancer-initiating cells [50]; Msi1-expressing cells are identified as key drivers of pancreatic cancer [51]; Sox2-expressing cells are identified as tumor-propagating cells and show cancer stem cell (CSC) property in squamous-cell carcinoma [52]; Dclk1 labels intestinal tumor stem cells, which produce tumor progeny [53]. In these studies, CD133, Sox2, Msi1 or Dclk1, which are frequently used as CSC markers, are either typical markers for NSCs and/or their genes are specifically expressed in neural tissues in vertebrate embryos [23, 54–56] (Fig. 1A-D). Their use as markers of NSCs/NPCs has been extensively documented. These studies demonstrate that the markers label both NSCs/NPCs and tumor-initiating cells (TICs), suggesting an association between TICs and neural stemness. It is usually argued that neural stemness markers are sometimes also markers for other types of stem/progenitor cells. This issue will be discussed later.

Polyploidy formation by cancer cells, an important factor promoting tumor growth and metastasis, has been extensively investigated, and targeting polyploidy was suggested as a potential therapeutic strategy [59–63]. Polyploid giant cancer cells (PGCCs) exhibit stemness because they express...
OCT4, SOX2, NANOG, SSEA1 and CD133, and have the potential of differentiation into cell types of all three germ layers in vitro [60, 64]. Although Oct4/OCT4, Sox2/SOX2 and Nanog/NANOG are typical markers for both mouse and human ESCs, their expression is enriched or localized to neural cells during vertebrate embryogenesis [23, 57, 58] (Fig. 1A, E, F). SSEA1 is not a marker for human ESCs, but rather a marker for early NSCs [65]. Marker expression suggests that the polyploid cancer cells are similar to NSCs. Moreover, NSCs also possesses the ability to generate fused polyploid cells in culture [66]. Thus, polyploidy formation provides an additional evidence for the similarity between cancer cells and NSCs.

**Tumor Phenotypic Heterogeneity**

A tumor is composed of heterogeneous populations of cells. How the heterogeneity is generated is also a complex issue [2, 3, 67–69]. In general, two major models have been proposed. One is the clonal evolution model, in which heterogeneity is explained as a result of natural selection. This model emphasizes the central role of genetic heterogeneity arising from a Darwinian-like evolution in phenotypic heterogeneity. Nevertheless, how genetic heterogeneity causes phenotypic heterogeneity seems to be not understood at all. It has not been confirmed that phenotypic heterogeneity is directly derived from genetic heterogeneity. It is challenged by the CSC model, in which a subpopulation of cancer cells has the ability of indefinite self-renewal, initiating tumor formation and driving tumor growth. In this model, heterogeneity is generated by differentiation of CSCs [5, 67, 70, 71], comparable to the tissue hierarchy generated by normal stem cells. However, it has not been characterized whether CSCs in different cancers exhibit the stemness of a similar type of stem cells, or CSCs in each cancer exhibit the stemness of the stem cells of the respective tissue of tumor origin, or CSCs are of a mysterious character and not comparable with any known stem/progenitor cell types. CSCs are also termed as TICs, and generally identified with certain markers. However, CSCs identified with markers might represent just a subpopulation of TICs, since CSCs are also heterogeneous cell populations. For an instance, CD133 is one of the most frequently used CSC markers. Both CD133+ and CD133− metastatic cancer cells initiate tumors in colon cancer [72], and CD133+ and CD133− cells from primary glioblastoma seem to be equally tumorigenic [73]. Hence, TICs might be a more generalized expression for the cells capable of tumor initiation, regardless of the presence or absence of particular CSC markers. TICs form tumors in nude mice. Nevertheless, how tissues or cells are differentiated in xenograft tumors had not been examined carefully except teratomas formed by transplanted ESCs, iPSC or teratocarcinoma cells. In our study, we observed that xenograft tumors formed by cancer cells contain tissue or cell types derived from three germ layers, as revealed by tissue-specific gene expression assay and immunohistochemistry assay with specific markers [39]. Tissue or cell differentiation from different lineages in xenograft tumors formed by TICs is rather similar to the xenograft tumors formed by NSCs/NPCs. Tumors of primNSCs are characteristic of teratoma/teratocarcinoma because they contain well differentiated tissues with readily identifiable morphology. Tumors from E9 NSCs and E13.5 NSCs/NPCs are less abundant in tissue diversity. Nevertheless, expression of markers for ectodermal, endodermal and mesodermal lineages was all detected. Similarly, xenograft tumors from cells of different cancers, for example, melanoma, colorectal carcinoma or glioblastoma, show significant expression of lineage markers or contain cells from different lineages, including SOX1-positive cells and MAP2-positive cells derived from ectoderm, ACTA2- and BGLAP-positive cells derived from mesoderm, AFP-positive cells derived from endoderm. These cell types were also present in tumors derived from Myod1 knockout myoblast cells [39], meaning that blocking of a pro-differentiation factor leads to loss of myoblast identity, gain of neural stemness and cell tumorigenicity, and re-acquisition of differentiation potential. Besides these findings, pluripotent differentiation potential of particular type of cancer cells, the teratocarcinoma cells and their capability of contribution to formation of chimera embryos have been observed in nearly a half century ago [74, 75]. Although CSCs have been described and delicate molecular mechanisms for establishing CSC phenotypes have been proposed in numerous publications, the nature of CSCs has not been characterized. Consequently, it is not known whether CSCs could differentiate along the differentiation lineage of a tissue stem/progenitor cell or the lineages of other types of stem/progenitor cells. In fact, in vitro generated cells with CSC properties have the potential to differentiate into cells expressing markers for neuron, endothelial cells and muscle [76]. CSCs in glioblastoma can differentiate into tumor-endothelium or vascular pericytes [77–79]. Colon cancer stem cells reveal a multi-lineage differentiation capacity [80]. These cells do not follow the differentiation hierarchy of a particular type of tissue stem cells along a fixed lineage. In summary, cancer cells capable of tumor initiation, or TICs, exhibit property of neural stemness and have the potential of differentiation into diverse tissue or cell types, contributing to intra-tumor phenotypic heterogeneity. The phenotypic difference between tumors has been a primary focus of tumor research. In fact, there exist many cell or tissue types in common among different types of tumors. In each tumor, there are immature neuroepithelial-like cells that function as TICs and express neural stemness markers, such as SOX1-positive cells. Indeed, NSCs can be derived, at least, from human teratomas [81]. Tumors also
contain neuron-like cells or nerves [28–31]. Cells expressing smooth muscle protein ACTA2 are widely detected in different tumors. AFP has been used as a marker for cancers of the liver, testicles, and ovaries, and is expressed also in other types of cancers, such as colorectal and gastric cancers [82–84]. Moreover, pathological studies reveal similar tissues or cells in different tumors. For example, osteoclasts are multinucleated giant cells that are responsible for bone dissolution and absorption. They are not present in normal tissues other than bones. Similar cells have been found in tumors of pancreas [85–89], liver [89–93], skin [94–97], lung [98–102], breast [103–107], and in many other tumors. Osteoid and bone formation was found in different cancers, such as breast cancer and melanoma [108, 109], etc. Thus, under the appearance of big phenotypic difference between tumors, there are in fact many tissue or cell types in common.

The intra- or inter-tumoral heterogeneity should be a result of differentiation potential of TICs, under the control of intra- and extracellular signals. Differentiation potential of TICs should also account for cancer types that appear irrelevant with the tissue of origin, e.g. primary osteosarcoma of the breast [109] and the ‘muscle cancer’ (rhabdomyosarcoma) from non-muscle cells [110], for which the mechanisms have been unknown. Teratomas/teratocarcinomas are considered as a special type of tumor since their initiation and progression is similar to a chaotic process of embryonic development, which appears rather different from other types of tumors. Nevertheless, the pluripotent differentiation potential of NSCs and TICs suggests an intrinsic link between teratomas/teratocarcinomas and other types of tumors. TICs of teratomas/teratocarcinomas should be more similar to primNSCs in differentiation potential, producing a tumor of a disorganized mixture of well-differentiated tissues or organs. The differentiation potential of TICs of other types of tumors might be more defected due to extensive genetic, epigenetic and microenvironmental variations, in particular the defect in pro-differentiation or tissue-specific genes, leading to tumors with more severely defected cell or tissue differentiation. These tumors can be considered as degenerated or more severely defected forms of teratomas/teratocarcinomas. Cancer was ever proposed as a developmental disorder or as ‘development gone awry’ [111] (and references therein).

**Neural State as the Ground State for Tumorigenicity and Pluripotent Differentiation Potential**

The earliest demonstration of the link between cell tumorigenicity and pluripotent differentiation potential was conducted on embryonic carcinoma cells (ECs) derived from teratocarcinoma. After the finding of ECs in more than a decade later, mouse ESCs were isolated and the properties of ESCs were observed to be very comparable with those of ECs. Both cell types form teratomas and display pluripotent differentiation potential [112]. From historical view, it was a type of cancer cells that inspired the study of pluripotent property of ESCs. It was postulated that tumorigenicity and pluripotency are two coupled cell properties [113, 114]. Characterization of iPS was also dependent on the two cell properties, which is usually performed with xenograft tumor formation assays in nude mice. However, why should pluripotent cells be tumorigenic has been largely unknown. Our recent study and studies by others suggest that the neural ground state is the link between cell tumorigenicity and pluripotent differentiation potential.

The default fate of embryonic pluripotent cells is neural. Blocking endogenous factors in somatic cells also leads to the gain of a neural property at the cost of original cell identity [33, 39, 115]. This means that either embryonic pluripotent cells or somatic cells turn into neural cells when appropriate endogenous factors are blocked. The effect reflects that neural state might be the ground state of cells, upon which all cells are formed. The question is, why the property of neural stemness, but not of other cell types, is the ground state of tumorigenicity and differentiation? The answer resides in evolution. Among the three germ layers, origin of ectoderm is the earliest during evolution, followed sequentially by endoderm and mesoderm [116]. Analysis of neural genes and non-neural genes in the ectoderm showed that one peak of emergence of neural genes is already present in the time point representing the last common ancestors (LCA) of eukaryotes and the other is at the time of emergence of the eumetazoan [116], indicating that genes relevant for neural development have an earlier evolutionary origin. Metazoans are all evolved from unicellular ancestors, an earlier origin suggesting that neural genes might play a critical role in multicellularity. *Monosiga brevicollis*, *Amphimedon queenslandica* and *Trichoplax adhaerens* are the closest species representing transition from unicellularity to multicellularity, and share a last common unicellular ancestor in more than 600 million years ago. *M. brevicollis* represents the closest unicellular relatives of metazoans [117]. *A. queenslandica* represents the oldest surviving metazoan and an evolutionary intermediary between unicellular choanoflagellate protists and eumetazoans, and *T. adhaerens* is the basal eumetazoan species. Interestingly, a majority of genes with enriched expression in vertebrate embryonic neural tissues can be traced back to these species [39], suggesting that the founders of most neural genes have emerged during the transition from unicellularity to multicellularity. More importantly, more than 60% of genes in *M. brevicollis* that are homologous to vertebrate genes are genes enriched in embryonic neural cells. The overwhelming presence of founders of vertebrate neural genes in the closest unicellular relative of metazoan implies that the last common unicellular
ancestor is biased towards a neural state [39]. The vertebrate homologous genes in *M. brevicollis* are mostly involved in organelle part, intracellular part, cytoplasm, cytosol, etc., that are basic functional constituents common to all animal cells, such as ribosomes and proteasomes. Moreover, these genes are mainly associated with regulation of cell cycle, metabolism and gene expression. This means that a neural biased state in the unicellular ancestors was the beginning state or ground state of multicellularity, and this state was an adaptation of unicellular ancestors to the low oxygen environment. Coherently, the intrinsic property of adaptation of neural state to hypoxic environment is reflected by that low oxygen condition enhances the property of neural stemness and the generation of NSCs from fibroblasts [115, 118]. The evolutionary advantage of neural state laid the foundation for the evolution of the most complex system in higher animals, the nervous system.

The neural ground state is also reflected by that the machineries required for basic cellular physiological functions and developmental programs are mostly enriched in embryonic neural cells. Here are some examples. Expression of genes promoting cell cycle is enriched only in neural cells during embryogenesis [23, 119, 120] (Fig. 2A), meaning that embryonic neural cells undergo faster cell growth and proliferation than non-neural cells. Sufficient ribosome biogenesis and increased overall protein production is critical for cell growth [121, 122]. A faster cell cycle means a requirement for more protein synthesis. Correspondingly, expression of genes for the machinery of ribosome biogenesis and the machinery for protein translation is also enriched in embryonic neural cells [23, 123–126] (Fig. 2B, C). During normal neural development, ribosome biogenesis is downregulated with increased differentiation of NSCs in mouse embryos [127], an indication of higher requirement for ribosome biogenesis in more proliferative and fast growing cells. The machinery for protein quality control and turnover should match the situation of fast protein production to guarantee protein homeostasis for cell growth and proliferation. Coherently, genes for the components of proteasome, which is responsible for protein degradation, show neural biased expression in *Xenopus* or zebrafish embryos [128–130] (Fig. 2D). Alternative splicing has been proposed to underlie phenotypic novelty during evolution [131]. It contributes to developmental programs and plays critical roles in controlling cell differentiation, lineage determination, tissue or organ formation and homeostasis [131, 132]. Genes for the components of RNA splicing machinery are expressed dominantly in embryonic neural cells [133–135] (Fig. 2E). This neural dominant expression is in agreement with that neural genes are enriched in long genes, which contain more exons and introns than those in non-neural genes [39] (Table 1). Higher alternative splicing activity generates more transcripts that demand more machineries like ribosomes and proteasomes. Neural enriched expression during embryogenesis has been also observed for most of genes for epigenetic modification factors, including histone lysine deacetylases *hdac1-3*, histone acetyltransferase *crebbp*, DNA methyltransferase *dnmt1*, lysine methyltransferases *setdb1-dot1l*, lysine demethylases *kdm1a-kdm6b*, arginine methyltransferases *prmt1-prmt7*, and the putative arginine demethylase *jmjd6* [23, 136–142] (Fig. 2F). Epigenetic modification factors are involved widely in early embryonic development, maintenance of stemness and differentiation of stem cells, etc. An additional example is the neural dominant expression of the factors that reprogram somatic cells into pluripotent stem cells and of the reprogramming co-regulators [143] that improve the efficiency of reprogramming [23, 57, 58, 119, 139, 144–158] (Fig. 2G, H). Fast cell cycles means rapid DNA replication in cells, and consequently a higher probability of DNA damage and mutation. To avoid this, transcription of genes for the machinery of DNA damage and repair is enriched in embryonic neural cells [57, 147, 159–161] (Fig. 2I). Embryonic neural enrichment can also be found for other basic machineries. Enrichment of the genes for these machineries for basic cellular physiological functions implies that they should exist in a high level and function concertedly to meet the requirement by the fast-growing, highly proliferative, and differentiation potential of embryonic neural cells or NSCs. The neural biased expression is in contrast to that these genes should be uniformly expressed, as usually thought. These machineries do exist in other cell types, but usually at a lower level. Interestingly, a major part of genes for these basic machineries have a unicellular origin [39], suggesting that the embryonic neural cells represent the direct descendant cells of the unicellular ancestors.

Accordingly, fast cell growth and proliferation is a typical feature of cancer cells, and genes promoting cell cycle are usually upregulated during and promote tumorigenesis. Similar to the situation in embryonic neural cells, cancer cells show elevated levels of ribosome biogenesis, which plays a promoting role in tumorigenesis [122, 162–164]. Translation initiation factors are also usually upregulated in cancers and correlate with disease progression and poor prognosis [165]. Moreover, cancer cells also show higher levels of proteasomes and proteasome activity for protein quality control, so as to promote their survival, growth and metastasis [166–168]. Splicing factors function as both oncoproteins and tumor suppressors, depending on the functions of spliced variants they generate, but mostly being upregulated in cancers and functioning as oncoproteins [169, 170]. Dysregulated epigenetics is also a typical feature of tumorigenesis. Epigenetic modification factors are mostly upregulated and play primarily a promoting role in cancers, which has been reviewed extensively [171, 172]. Moreover, reprogramming factors and co-regulators are also generally
cancer-promoting factors, primarily contributing to the gain or maintenance of stemness in cancer cells. Genes involved in DNA damage and repair are often dysregulated or mutated in cancer. The inhibitors of PARP1/2, which play a key role in DNA repair, have been used for preclinical and clinical trials [173, 174]. Therefore, similar to the situation in embryonic neural cells or NSCs, these basic machineries should be coordinately upregulated, such that they can be expressed in a higher level to satisfy the higher requirements by cancer cells than by normal tissue cells. Enrichment of the basic machineries of cellular physiological functions and developmental programs in embryonic neural cells or NSCs consolidates that neural stemness represents a ground or basal state upon which other cell types are derived. These basic machineries should be repressed in response to differentiation, leading to a reduced level in differentiated states.
cells. When a differentiation signal is removed/repressed in a cell, like what occurs during tumorigenesis, the cell will turn back to the ground state, a highly proliferative and fast-growing state that needs more machineries for cell cycle, protein synthesis, translation, degradation, splicing, etc.

According to gene conservation and expression pattern analysis, it is suggested that neural-biased unicellular state is the beginning state of multicellularity and cell type diversification [39], emergence of non-neural state requires inhibition of the initial or ground state. In other words, neural cells are formed by an evolutionarily predetermined ground state and non-neural cells are induced upon the neural ground state. Induction of non-neural cells needs repression of neural ground state by non-neural pro-differentiation signals. Accordingly, genes enriched in embryonic neural cells, such as those discussed above, are downregulated or repressed in non-neural cells, ultimately leading to the reduction of cell tumorigenicity and pluripotent differentiation potential. Besides evolutionary advantage, neural genes are characteristic of over-representation of long genes with more exons and introns, as compared with non-neural genes [39, 175, 176], with the average length of neural genes in general being about two times of the length of non-neural genes [39] (Table 1). Longer genes facilitate binding of different regulators, generating more spliced variants or forming secondary or tertiary structures. This means that longer genes can serve as more flexible scaffolds for regulatory signals required for generation of diverse types of cells. Shorter genes should have less flexibility. In this regard, enrichment of longer genes may confer neural stemness the plasticity and make it a more appropriate initial state for cell differentiation.

During tumorigenesis, tissue-specific genes are usually downregulated or silenced in cells. This would cause derepression of neural ground state and facilitate somatic cells to return to the ground state, the most direct route that needs no instructive signals. The resulting cells acquire the property of neural stemness, the capability of self-renewal and differentiation into cells of different lineages. An atavistic effect of tumorigenesis has been suggested because cancer is driven by ancestral gene regulatory networks, and cancer genes are mostly conserved in unicellular and basal species of multicellular organisms [177–181]. Moreover, cancer development is suggested as a reverse evolution from multicellularity to unicellularity [182, 183]. The metabolic style in cancer cells (or more precisely, TICs) also reflects the characteristics of metabolism of the last common ancestor of unicellular and multicellular organisms in an oxygen-deficient environment. Like in cancer cells, NSCs and pluripotent stem cells (PSCs) rely mainly on aerobic glycolysis as well [184–187]. PSC differentiation into NSCs does not change or even increases glycolysis, whereas PSC differentiation into mesoderm and endoderm or NSC differentiation into neurons decreases glycolytic flux [185, 186]. Reprogramming of somatic cells into pluripotent state causes metabolic shift from oxidative phosphorylation to glycolysis again [187]. The similarity in metabolic styles between tumorigenic cells and NSCs is also reflected by that the factor regulating neural development reprograms metabolism in cancer, and confers a stemness style of metabolism in cancer cells [188–190]. Cancer cells are characteristic of genomic instability and gene mutations, which are also the feature of the genomes of unicellular species under adverse environment [191, 192]. Coincidentally, both NSCs and PSCs are prone to genomic instability [193, 194]. As mentioned above, enrichment of longer genes with more splice variants is a unique feature of neural cells, and this enrichment is also present in pathways linked to cancers [195]. These shared features, as summarized in Table 2, indeed reflect an intrinsic link between neural stemness, cell tumorigenicity and pluripotent differentiation potential.

**General Stemness Versus Specific Stemness**

Amphibian blastula ectodermal cells and mammalian ESCs are derived from cells that appear at cleavage stage of embryogenesis, and considered as the basis for differentiation. By contrast, NSCs are considered as a type of tissue stem cells because they emerge later than pluripotent embryonic cells, are derived from ectoderm, and exhibit differentiation potential for neural lineage during normal embryonic development. Therefore, change of ESCs into primNSCs is considered as a differentiation effect and loss of pluripotency. However, based on the analyses above, this change might be more logically interpreted as an effect of restoration of neural ground state of ESCs. The induced pluripotency should be per se also an effect of gain of neural stemness in somatic cells. As shown in Fig. 2G and H, the four original reprogramming factors, Sox2, e-Myc, Oct4 and Klf4, and subsequent alternative reprogramming factors and reprogramming co-regulators are enriched in neural precursor/progenitor cells during vertebrate embryonic development, in addition to their earlier expression in the inner cell mass of mammalian blastocysts. Functionally, Oct4 homologous proteins in *Xenopus* are required for neural induction and promote neuroectodermal gene expression [197, 198]. Sox2

**Table 1** Difference in average gene length and exon/intron numbers between neural and non-neural genes (From Ref. [39])

|                         | Neural enriched genes | Non-neural genes |
|-------------------------|-----------------------|------------------|
| Average gene length     | 92,765                | 42,911           |
| (nucleotides)           |                       |                  |
| Average number of exons/| 13/12                 | 9/8              |
| introns per gene        |                       |                  |

The table shows that neural genes are longer and have more exons and introns compared to non-neural genes.
Enriched in long genes with more splice variants [39, 175, 176]

Pluripotent differentiation potential [17, 39, 43]

Characteristic of aerobic glycolysis. Differentiation into neurons decreases glycolysis [184, 185]

Tumorigenic progenitor cells [References]

Cancer cells [References]

Pluripotent stem cells (PSCs) [References]

Tumorigenic [39]

Dependence on activation of ancestral regulatory networks [177–180]

Neural stemness as the default state of PSCs [9, 16–21]

Neural stemness [23, 25, 39]

Tumorigenesis as a process of reverse evolution and also a process of gain of neural stemness [39, 178, 181–183]

Neural stemness as the default state of PSCs [9, 16–21]

Unknown

Pluripotent differentiation potential [39, 74, 75, 112]

Pluripotent differentiation potential

Characteristics of aerobic glycolysis. Differentiation into NSCs does not change or increases glycolysis; differentiation into mesoderm and endoderm decreases glycolysis [185, 186]

Determined by neural biased state of last common unicellular ancestors [39]

Unicellular origin of pluripotency [196]

Neural stemness

Unknown

Enriched in long genes with more splice variants [39, 175, 176]

Unknown

Table 2 Comparison of the properties of three types of cells

in neural development has been well documented. In particular, the four reprogramming factors for generating iPSCs also induce NSCs/NPCs from fibroblasts [199]. Likewise, neural crest cells share many key regulatory factors with blastula animal pole cells during Xenopus embryogenesis, and correspondingly, neural crest cells retain pluripotent differentiation potential as in blastula animal pole cells [144]. The idea that neural crest is considered as a fourth germ layer also emphasizes the differentiation potential of neural crest cells into different lineages [200]. Taking the evolutionary advantage of neural state into consideration, reprogramming of somatic cells into a pluripotent state should be interpreted as a process of restoring neural ground state in somatic cells, consequently leading to the gain of pluripotent differentiation potential and tumorigenicity, a process similar to that occurs during tumorigenesis. TGFβ signaling is required for neural inhibition during germ layer differentiation and for maintenance of ESC pluripotency (i.e., preventing ESCs from adopting a neural fate) [201–203]. This means that TGFβ in ESCs represses the neural ground state on one hand and the other, functions to prime the potential for non-neural differentiation [204, 205]. As differentiation continues, factors promoting differentiation of a particular cell type should inhibit further the neural ground state and meanwhile confer the property of the cell type. This relationship can be traced back to the transition from unicellularity to multicellularity during evolution. As a prime signaling for cell fate diversification, emergence of TGFβ pathway was accompanied with the start of multicellularity [206]. The pathway is not present in M. brevicollis [207, 208], but present in A. queenslandica [208, 209]. This multicellular organism represents the last common ancestor to living metazoans and develops with the least level of cell type diversification. Cells with pluripotent state are present in A. queenslandica, and pluripotency has a unicellular origin [196], which might be biased toward a neural state [39]. Therefore, both our and previous studies by other groups support that neural stemness should represent the general stemness [39, 43].

The similarity in regulatory networks and cell properties between ESCs and primNSCs and the temporal order of cell differentiation during embryogenesis suggest that ESCs are most closely related with primNSCs. It is not surprising that most markers for ESCs or pluripotency are also markers for NSCs/NPCs or enriched in embryonic neural cells. With the progression of differentiation, stemness decreases gradually, until possibly a total loss in fully differentiated cells. There are somatic stem/progenitor cells in adult animals that are required for tissue regeneration and repair. These specific stem cells also display the properties like proliferation and self-renewal, but have restricted differentiation potential along a fixed lineage. They represent an intermediary between the general stemness and a maturely differentiated state. They derive the properties of stemness from the general stemness, and meanwhile are conferred with the specific property of a lineage by lineage-specific regulatory factors. The association between general (or neural) stemness and the stemness of somatic tissues can be manifested by that many markers for NSCs/NPCs are also markers for somatic stem cells. For example, neural stemness markers BMI1, MS11, SOX9 and CD133 can be used for labeling intestinal stem cells [210]; the NSC marker NESTIN also identifies bone marrow mesenchymal stem cells [49], hair follicle sheath progenitor cells [211], testicular stem Leydig cells
Tumorigenesis and Regeneration

The capability of regeneration is inversely correlated with the evolutionary positions of animals, with higher regenerative capacity in lower animals and lower capacity in higher animals [216]. Moreover, animals at early developmental stage usually exhibit stronger regenerative capacity. There is also an inverse correlation between the propensity for regeneration and the capacity of tumorigenesis. It seems that animals with the ability of regeneration of complete limbs or head rarely develop cancer [217]. Dedifferentiation has been suggested as one of the major routes to regeneration [216, 218]. Some regeneration events are mediated by cell fusion, which eventually induces also an effect of cell dedifferentiation and proliferation [216]. Cell fusion-mediated polyploidy formation and dedifferentiation in cancer cells was discussed above. Dedifferentiation will cause the reversal of differentiated state into an undifferentiated state that is determined by neural ground state, and re-acquirement of differentiation potential. An example is the dedifferentiation of myoblast cells in response to loss of a muscle differentiation factor Myod1 [39]. Moreover, dedifferentiated fat cells display the potential of differentiation into multiple lineages, including neural lineage [219]. Due to the increasing complexity of gene regulatory networks of cell differentiation during evolution, it can be postulated that cells in animals at lower evolutionary positions or at early developmental stages are more easily to dedifferentiate, and the dedifferentiated cells are more easily to re-differentiate under the control by a relatively simple regulatory network. By contrast, cells in higher animals or at adult stages are more difficult to dedifferentiate and re-differentiate because more sophisticated regulatory networks are involved. Consequently, higher animals have a low capacity of regeneration because of more complicated regulation of dedifferentiation and re-differentiation, which might have higher possibility of dysregulation. When dedifferentiate and re-differentiate are not regulated properly, cancer could occur because of the tumorigenicity of dedifferentiated cells.

The Current Model and Other Models of Tumorigenesis

The analysis above indicates that tumorigenesis follows a general rule beneath the complicated genetic and phenotypic heterogeneity of tumors. Cells may experience different changes due to suffering from intracellular/extracellular insults, including mutations, chromosomal instability, aneuploidy, microenvironmental changes, gene misregulation, etc. Any single change is hard to explain the complexity of tumorigenesis. Whatever the insults are, the ultimate consequences should be the change in gene expression in cells. When tissue-specific genes or differentiation genes are accidentally downregulated/silenced or neural genes are upregulated/activated or both, the result will be the loss of original cell identity and gain of property of neural stemness and tumorigenicity. Gain of property of neural stemness means the capability of self-renewal and differentiation into different tissue/cell types, which contribute to tumor phenotypic heterogeneity. This is a default route that is pre-determined by evolution. The last common unicellular ancestors of metazoan had a neural biased state, the initial or ground state for multicellularity. Emergence of novel genes and regulatory networks was required for inhibition of neural state to generate non-neural cells in metazoan. TGFβ signaling, which is critical for maintaining ESC pluripotency and neural inhibition, began to emerge in the basal species of metazoan such as A. queenslandica. It might be the earliest signal for diverting the neural state to a non-neural state, as it functions to prime the ESCs the potential to non-neural differentiation. Cells with pluripotency exist in A. queenslandica, which are most closely related with the unicellular relatives of metazoan [196]. With the progression of metazoan evolution, regulation of cell differentiation becomes more complex, the property of neural stemness, the differential and tumorigenic potential in the cells is further decreased. Repression of differentiation signals will cause de-repression of neural ground state, leading to the gain of neural stemness, differentiation potential and tumorigenicity in either embryonic pluripotent cells (hence the ‘neural default model’) or somatic cells. This process of reverse evolution is a default route without the necessity of inducing signals, and is the most direct route hence for tumorigenesis. The proposed concept is illustrated in Fig. 3.

The property of neural stemness is hopefully able to integrate different characteristics of tumorigenic cells and tumorigenesis. As summarized in Table 2, NSCs/NPCs and tumorigenic cells share regulatory networks, both exhibit neural stemness and differentiation potential; both are dependent on expression or activation of ancestral genes (the atavistic effect); both rely primarily on aerobic glycolytic metabolism; both have the potential to differentiate into various cells or tissues that are derived from all three germ layers,
resembling severely disorganized or more severely degenerated form of embryonic development; both are enriched in longer genes with more splice variants that might provide more plastic scaffolds for cell differentiation, etc. This might be a key point to understand tumorigenesis and pluripotent differentiation potential.

**Future Directions**

Tumorigenesis is a complex process that cannot be interpreted by individual genes, mutations or any other individual molecular events. High-throughput approaches, for example single-cell analysis [220], might unveil the minu-tiae within or between tumors. Nevertheless, how these finest details benefit the understanding of tumorigenesis and cancer therapy remain uncertain [221]. Now that neural stemness and the underlying regulatory networks is the ultimate determinant of tumorigenicity and differentiation potential, cancer research, or more generally, the research on pluripotency and differentiation, should benefit more from a focus on the property of neural stemness. It is no doubt that great progress has been made on cancer therapy, in particular the targeted therapy and immunotherapy. In both strategies, the spectrum of cancers that benefit from the therapy is quite narrow, and the therapeutic efficiency is usually compromised or lost by chemoresistance or immunoresistance [6, 222–227]. Hyperprogression of cancer even occurs in patients under immunotherapy [228, 229]. These effects in turn stimulate the waves of exploration of the underlying mechanisms, a task seemingly not easier than understanding cancer itself [6, 222–227]. Targeted therapy and immunotherapy are dependent on the presence of particular target molecules, which are not necessarily distributed uniformly in all tumorigenic cells during cancer progression because novel mutations, signal feedbacks, etc., are constantly emerging. This is like that chopping off a branch of a fast growing tree usually does not cause a significant damage to the tree’s life. An alternative strategy to overcome these difficulties might be targeting the kernel property of tumorigenic cells, the neural stemness, which can be achieved by differentiation. Loss of neural stemness in tumorigenic cells via neuronal differentiation causes suppression of malignant features and tumorigenicity of tumorigenic cells [23, 39]. Suppression of neural stemness can also be achieved by enforced expression of non-neural pro-differentiation factors in tumorigenic cells. During normal embryogenesis, differentiation factors of different tissues or organs should inhibit each other, thereby maintaining the integrity of different cell types and establishing boundaries between different tissues/organisms. It can be deduced that non-neural pro-differentiation factors should be able to repress neural stemness, and consequently, suppress cell tumorigenicity. As reported, pancreatic cancer cells can be converted into acinar cells by Ptf1a, a critical protein required for pancreas development and differentiation, leading to suppression of tumorigenesis in pancreas [230]. Converting breast cancer cells into post-mitotic adipocytes can inhibit metastasis of breast cancer [231]. Targeting neural stemness via differentiation causes reprogramming of the regulatory networks underlying tumorigenicity of cancer cells. The genome-wide change might bypass the complex signal feedback loops that cause resistance in targeted therapy.

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