Review

Special Issue: Celebrating 30 years of biotechnology

Stem cell metabolic and spectroscopic profiling

Paul Ramm Sander[1,2], Peter Hau[2], Steffen Koch[3], Karin Schütze[3], Ulrich Bogdahn[2], Hans Robert Kalbitzer[1], and Ludwig Aigner[4,5]

[1] Institute of Biophysics and Physical Biochemistry, University of Regensburg, 93040 Regensburg, Germany
[2] Department of Neurology, University of Regensburg, Universitaetsstrasse 84, 93053 Regensburg, Germany
[3] CellTool GmbH, Am Neuland 1, 82357 Bernried, Germany
[4] Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Strubergasse 21, 5020 Salzburg, Austria
[5] Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, Strubergasse 21, 5020 Salzburg, Austria

Stem cells offer great potential for regenerative medicine because they regenerate damaged tissue by cell replacement and/or by stimulating endogenous repair mechanisms. Although stem cells are defined by their functional properties, such as the potential to proliferate, to self-renew, and to differentiate into specific cell types, their identification based on the expression of specific markers remains vague. Here, profiles of stem cell metabolism might highlight stem cell function more than the expression of single genes/markers. Thus, systematic approaches including spectroscopy might yield insight into stem cell function, identity, and stemness. We review the findings gained by means of metabolic and spectroscopic profiling methodologies, for example, nuclear magnetic resonance spectroscopy (NMRS), mass spectrometry (MS), and Raman spectroscopy (RS), with a focus on neural stem cells and neurogenesis.

The different flavors of stem cells

Stem cells are defined by their potential to divide, to self-renew, and to generate different mature cell types. They have been identified in almost every organ and tissue of the human body. Besides hematopoietic stem cells (HSCs), which have been in clinical use for years, mesenchymal stem cells (MSCs), neural stem cells (NSCs), and also embryonic stem cells (ESCs) are currently tested in preclinical and clinical settings for their potential to regenerate and repair damaged tissues (Box 1). ESCs are pluripotent and give rise to all cell types of the body. Later in development, stem cells become more and more restricted in their differentiation potential and generate germ layer and/or organ-specific cells and are therefore defined as being multipotent [1]. However, more recent data clearly suggest that stem cells might cross lineage boundaries. Moreover, the identity of stem cells, despite tracing their presence and fate through genetic reporter models, is in many cases still a matter of debate. For example, although NSCs in the adult brain have long been considered as immature cells, it is now evident that specialized astrocytes in the neurogenic regions comprise the neural stem cell pool [2]. Moreover, the fact that stem cells might be present in different stages of activation such as in quiescence or in the cell cycle enormously complicates the issue of stem cell identity. This is currently well described in the adult neural stem cell niche, where stem

Glossary

Metabolomics: in contrast to targeted approaches, metabolomics constitutes a more holistic analysis of metabolism by means of profiling techniques (e.g., NMRS, MS, and optical spectroscopy). Typically, hundreds or thousands of metabolites are analyzed simultaneously. Besides biofluids (e.g., urine, blood, liquor), metabolomic approaches can be applied to cell culture (e.g., supernatants, cell extracts/lysates, or whole cells) and *in vivo* (localized NMRS at clinical MRI scanners).
MLs: constitute a subgroup of cellular lipids whose macromolecular structure is flexible enough – unlike rigid membrane lipid assemblies – to permit NMRS detection. Predominantly associated to cytoplasmic lipid droplets, these NMR-visible lipids can be found in various cell and tissue types, especially under conditions of cellular stress (e.g., confluence, pH stress, apoptosis, and necrosis).
MS: due to its high sensitivity and resolution, MS is the most common profiling technique employed in metabolomic studies. It is based on resolvable differences of mass-to-charge ratios of ionized molecules in electromagnetic fields. However, relatively high chemical preprocessing demands prevent *in situ* or *in vivo* applications.
NAA: is a metabolite predominantly found in neurons that can be detected even at clinical MRI scanners with low sensitivity due to its high abundance (e.g., within the healthy brain). Although its function has not been clarified in detail, in clinical routine, it is used as a marker for functional neurons – and for all pathologies that lead to a decrease of density of functional neurons.
NMRS: following MS, NMRS is the second most common metabolomic technique. Based on molecular fingerprints due to chemical shifts of resonance frequencies of nuclear spins within a strong magnetic field, NMRS can also be applied *in vivo* (e.g., at clinical MRI scanners), to detect changes within the metabolome noninvasively. *In vitro*, the straightforward sample preparation without major chemical preprocessings permits convenient high-throughput studies. However, the relatively low sensitivity implicates a high sample quantity and limits the detectability/quantifiability of scarce metabolites.
PCA: is a common algorithm to reduce high-dimensional data (e.g., metabolic profiles). The data space is projected onto a new space of linear combinations of the data and ordered according to the highest statistical variability.
PLS-DA: is a regression analysis that projects high-dimensional data onto a new space in which the data optimally fit a predefined linear model.
RMS: RMS is a combination of RS and microscopy. It enables molecular imaging with high spatial resolution by means of gating to defined spectral regions specific for, e.g., RNA or proteins.
RS: RS is an optical technique based on inelastic scattering. Due to its high sensitivity and spatial resolution, it enables noninvasive metabolomic studies on a single-cell level. However, the spectral resolution is limited.
SVM: SVMs are supervised data reduction algorithms that optimize clusterings of high-dimensional data according to predefined groups.

Corresponding author: Aigner, L. (ludwig.aigner@pmu.ac.at).
Keywords: stem cells; metabolomics; NMR spectroscopy; mass spectrometry; Raman microspectroscopy; mobile lipids.

 0167-7799/$ – see front matter http://dx.doi.org/10.1016/j.tibtech.2013.01.008

Box 1. Translation to the clinic

Normal or diseased stem cells offer potential for therapies based on regeneration, therapeutic targeting, and drug delivery. A clear correlation of the single- or oligo-cell metabolome to certain cell types, states of differentiation, and therapeutic responses could be of immense value for translational approaches.

Stem cell implantation has been used in neurodegenerative diseases, such as Parkinson's disease and Huntington's disease [71–74], and myocardial infarction [75]. Although the difficulty in securing functional neurons prevents a broader application of transplants in Parkinson's disease, iPSCs may enable replacement therapies [76]. A routine NMRS follow up of stem cells, including markers of maturation and functionality, could allow monitoring the development of implanted or induced stem cells. Only preliminary data have suggested the value of NMRS in this context [77].

TICs may be relevant for the development and maintenance of solid tumors in humans [78,79]. Therefore, TICs could be valuable therapeutic targets. In glioblastoma, immunotherapeutic [80] and molecular targets could be envisioned, including the specific microenvironment, angiogenic signals, receptor tyrosine kinase-activated pathways, pathways involved in the neural stem cell development, specific transcription factors, and epigenetic modulators [81,82]. Several pathways induce specific alterations in the tumor metabolome [83], and targeting by specific small molecule inhibitors or other drugs could be monitored by *in vivo* NMRS [14,15,17]. In addition, a preference for a glycolytic and anaplerotic metabolism – the notorious Warburg effect – has been found in glioma [57] and TICs from other solid tumors. Approaches aiming at the glucose metabolism can be monitored by use of ^{13}C-labeled glucose [70], a method that can be applied in humans using specific NMRS scanners.

It has been shown that mesenchymal stromal cells [84], neural stem cells [85], or HSCs [86] migrate in experimental models with a tropism to diseased brain areas, for example, sites of hypoxia and invasive tumor borders [87,88], opening the possibility to deliver drugs to these areas. The migration of such drug-delivery cells and the release of drugs could be monitored [84], preferentially by NMRS.

A prerequisite for all the mentioned approaches would be an improvement of the available clinical NMRS methods, especially in respect to voxel size, with the goal of showing a broader spectrum of metabolites. However, it might be sufficient to track larger volumes if the ratio of relevant cells is sufficient [27]. Finally, NMRS could be combined with other clinical imaging tools [89] to characterize the features of stem cells, and their maturation and treatment, in a multifunctional approach.

cell proliferation in the long run depletes the stem cell niche during aging [3]. Furthermore, there is no clear distinction between stem cells and proliferating progenitor cells. Even more confusing, *in vitro*, 'stem cells' are often cultured under proliferation conditions, thus more closely resembling progenitor cells rather than stem cells, if at all [4]. Besides astrocytes acting as neural stem cells in the adult brain, there is an increasing amount of evidence for cellular plasticity in the sense of differentiated mature cells reverting back into stem cells. For example, pericytes in the brain have recently been shown to have mesenchymal as well as neural stem cell potential [5]. The most dramatic example of cellular plasticity is probably demonstrated by induced pluripotent stem cells (iPSCs). Somatic cells are reverted into pluripotent ESC-like cells by means of reprogramming using a small set of pluripotency genes such as Oct4, Sox2, c-myc, and Klf4 [6]. Similar to iPSCs, somatic cells or tissue-specific stem cells might spontaneously acquire a higher level of pluripotency, which however is regularly associated with an increasing tumor potential. These cancer stem cells (CSCs) or tumor-initiating cells (TICs) exist [7,8], however, again their ultimate identity remains elusive. This short summary on different stem cells and on stem cell plasticity clearly points to the complexity and to the difficulty of very central questions in stem cell biology such as stem cell identity and regulation of stemness. Moreover, it highlights the fact that there will neither be a prototypic stem cell gene or protein expression profile nor a prototypic stem cell metabolic profile, whereas there might be specific profiles for a particular activity stage of a particular stem cell.

Stem cell profiling

In contrast to targeted approaches of analyzing the expression of a small set of marker genes or the presence of a few metabolites, metabolic and spectroscopic profiling aims at a more holistic and integrated view into the metabolome. Typically, information on hundreds or thousands of molecules can be obtained simultaneously, in most cases either by MS (see Glossary) or by NMRS [9]. Although primarily applied to biofluids (urine, cerebrospinal fluid, or plasma), metabolic profiling of cultured cells has become a promising area of research [10]. The informative value of cell culture metabolomics is manifold, ranging from metabolic footprints, that is, consumption and release rates derived from supernatants/conditioned media [11,12], over metabolic fingerprints derived from cell extracts [10,13], to metabolic monitoring of intact cells [14,15]. Especially in the latter case, NMRS and Raman microspectroscopy (RMS) of living cells will greatly contribute to a better understanding of *in vivo* metabolism. Moreover, the prospect of translating *in vitro* findings to noninvasive *in vivo* studies, for example, by means of localized NMRS on preclinical [16] and clinical [17] MRI scanners, emphasizes the relevance of *in vitro* profiling approaches.

NMRS is a sophisticated and widely accepted high resolution method with a broad range of applications from, for example, the determination of protein structures to the monitoring of molecular reaction kinetics, and from high throughput metabolomics (e.g., blood, urine, liquor) [9] to the noninvasive detection of metabolites in humans at clinical MRI scanners [17]. NMRS is based on the effect of the chemical environment on the nuclear spins of a molecule and is reflected in small but resolvable shifts of the resonance frequencies when exposed to a strong magnetic field. The disadvantages of NMRS, that is, particularly the relatively high expenses and the limited sensitivity, are excessively compensated by its noninvasiveness, its potency of quantitative high throughput, and its atomic resolution. Moreover, the clinical approval and availability of modern MRI scanners makes NMRS a unique methodology with respect to a straightforward translation from bench to bedside.

By contrast, MS counters with very high sensitivity and resolution, accompanied by lower original and maintenance costs. It relies on the concept that differences in mass-to-charge ratios of ionized molecules can be resolved by means of electric and magnetic fields. The main drawbacks are relatively high chemical preprocessing demands and fairly low quantitative accuracy. Nevertheless, these drawbacks have not restrained MS from becoming the by far most widely employed methodology in the field of

metabolomics [9]. Moreover, due to its high sensitivity, even mass spectra of single cells could have been recorded [18].

Optical spectroscopy, also known as vibrational spectroscopy, has entered the field of cellular profiling only recently. The manifold methodologic flavors of optical spectroscopy can be grouped into Fourier-transform infrared spectroscopy (FTIR, based on absorption), RS (based on inelastic scattering), and coherent anti-Stokes Raman scattering (CARS) spectroscopy. To some extent, especially when combined with microscopy, that is, RMS and CARS microscopy, these methodologies might fill the gap between NMRS and MS by combining the advantages of both techniques, that is, the noninvasiveness of NMRS with the potential of single-cell analysis of MS [18]. Unfortunately, the relatively low spectral resolution renders the identification of the molecules giving rise to the spectral signals somewhat difficult. Therefore, in most cases, the specificity of optical spectra as a whole, sometimes called 'photonic fingerprint', is used as a qualitative rather than a quantitative measure of metabolites or other cellular molecules. Nevertheless, even coarse distinctions, for example, between RNA and proteins, proved to be of great value [19].

To extract relevant metabolic features out of the typically huge amount of data obtained by spectroscopic approaches, sophisticated multivariate pattern recognition techniques are essential [9]. For instance, a reduction of the data space to a small subset of linear combinations of the data containing the highest variability or yielding the optimum cluster separation, for example, by principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), or more sophisticated support vector machines (SVM) [20], has proven to reveal hidden relations, for example, between different metabolic pathways or between metabolic aberrations and pathologies.

However, apart from detailed methodological aspects of metabolic and spectroscopic profiling, which are beyond the scope of this review, and as already pointed out earlier, the definition of stem cells is most critical for metabolomics studies, because different stem cell aspects may lead to totally different metabolic and spectroscopic characteristics. As an extreme example, it is to be expected that the metabolic status of a quiescent stem cell differs substantially from a proliferating progenitor cell. Hence, caution has to be used when findings on energy metabolism, for example, are compared because different proliferation and function statuses may substantially alter metabolic profiles.

Therefore, findings on stem cell metabolism should be interpreted in terms of a cellular status that may be associated with certain aspects of stem cell characteristics rather than solely attributing these findings to the stem cell identity itself. Consequently, we review the most recent findings on metabolic and spectroscopic profiling focusing not only on cultured neural stem/progenitor cells but also on the intact brain, in particular on the developing brain, which constitutes a unique physiologic environment with highly abundant neural stem/progenitor cells that can be monitored noninvasively.

Specificity of the stem cell metabolome?

As the field of metabolic and spectroscopic profiling of stem cells has been entered only recently, the quantity of published data on specific features of stem cells remains limited. Most of the recent findings can be categorized into the following groups in arbitrary order (Table 1 and Figure 1): NMR-visible mobile lipids (MLs; stress-induced lipid droplets), choline-containing compounds (membrane turnover), amino acids (biosynthesis and anaplerosis), energy metabolism (glycolysis vs oxidative phosphorylation), the absence of maturation markers [e.g., N-acetyl aspartate (NAA) for neurons], and the relative abundance of RNA and macromolecules.

NMR-visible MLs and proliferative stress

NMR-visible MLs give rise to NMRS signals that can be found in unprocessed cells (Figure 2), tissues, and in the living organism [21–23]. Interestingly, in most cases, the appearance of these lipid signals is associated with cellular stress, for example, apoptosis, necrosis, pH decrease, confluence, and cytotoxic or static treatment. It was shown that MLs correlate to cytosolic lipid droplets [24,25]. The latest (yet not challenged) hypothesis about their origin links the imbalance between membrane lipid production at the endoplasmatic reticulum and reduced need at the membrane site (e.g., because of inhibited mitosis) to the accumulation of these surplus lipids as neutral, micrometer-sized droplets [26]. Surprisingly, one of these ML signals (the fatty acyl methylene peak at 1.28 ppm) has been attributed to neural progenitor cells (NPCs) *in vitro* and *in vivo* [27]. However, shortly after a couple of critical technical comments [28–30] and a subsequent response to these comments [31], we [32] and others [33] could show that – as for other cell types – MLs appear in NPCs upon cellular stress, for example, confluence (Figure 2) and apoptosis [32], and correlate to the percentage of apoptotic cells but not to stemness, which was assessed by comparing a variety of stem/progenitor cells at different developmental stages with respect to their NMR-visible lipid content [33]. Moreover, in glioblastoma brain tumor-initiating cells (BTICs), ML signals were detectable but neither correlated to clonogenicity, an *in vitro* hallmark of stemness, nor to CD133, one of the more reliable BTIC surface markers [14]. Instead, a significant correlation to the percentage of dead cells was again found. Indeed, a link between apoptosis and NPC/neurogenesis exists *in vivo* [34]. However, the robustness of the rather indirect detection of NPCs based on elevated apoptosis rates remains to be proved. Nevertheless, stem cell-specific responses of certain stimuli, for example, growth factors such as transforming growth factor-beta (TGFβ), may become manifest in specific ML modulations (own unpublished data). As a matter of fact, until now MLs have been found in several stem cell types at different developmental stages, including ESCs [33], NPCs [27,32,33], oligodendrocyte progenitor cells (OPCs) [27], and glioblastoma BTICs [14].

Choline and membrane turnover

Tightly related to membrane lipid synthesis and degradation, the metabolism of choline-containing compounds (total choline, tCho) also plays a crucial role in cell

Table 1. Findings on stem cell-specific metabolism

Findings	Stem cell types/tissues	Control cell types/tissues	Method	Refs
MLs				
In vitro				
Detectable in several stem/progenitor cell types; highest intensity in NPC	ESC, NPC (mouse), OPC (rat)	Neurons (rat), astrocytes, oligodendrocytes (mouse), macrophages (mouse), etc.	NMRS of cell suspensions	[27]
Not NPC-specific; increased upon confluence and induction of apoptosis	NPC (mouse), MSC (rat)	COS7 fibroblasts (monkey)	NMRS of cell suspensions	[32]
Detectable in ten different glioblastoma BTIC lines; correlation to cell death; no correlation to clonogenicity or CD133	BTIC (human glioblastoma)		NMRS of cell suspensions	[14]
Mainly in ESC, embryonic NSC, embryonic fibroblasts, and glioma cells; no significant signals in NSC differentiated from ESC or from IPC	ESC, NSC (mouse)	Differentiated NSC, astrocytes, neurons, glioma cells, melanoma cells (mouse)	NMRS of cell suspensions	[33]
Choline-containing compounds				
In vitro				
High tCho levels in ESC and NSC; in contrast to NSC and other cell lines, GPCho is absent and PCho most abundant in ESC	ESC, NSC (mouse)	From literature: neurons, glial cells, tumor cells (including meningioma, breast cancer, glioma)	NMRS of cell extracts	[42]
Decrease of ratio PCho/GPCho upon differentiation	ESC (mouse)	Differentiated ESC (mouse), carcinoma cells (F9)	NMRS of cell extracts	[43]
***Ex vivo* (rodent)**				
Decrease with rat brain maturation during the first 4 postnatal weeks	P1–P28[a] rat brain	Adult rat brain	NMRS, HPLC[b] of tissue extracts	[38]
Decrease with rat brain maturation during the first 3 postnatal weeks	P1–P21 rat brain	Adult rat brain	NMRS of tissue extracts	[39]
***In vivo* (rodent)**				
Decrease with rat brain maturation during the first 3 postnatal weeks	P1–P28 rat brain		*In vivo* NMRS	[37]
Increase with mouse brain maturation between week 4 and week 9	P10–P90 mouse brain		*In vivo* NMRS	[45]
***In vivo* (human)**				
Decrease with maturation of human fetal brain during gestational weeks 30–41	Human fetal brain		*In vivo* NMRS	[40]
Decrease with maturation of human fetal brain during gestational weeks 22–39	Human fetal brain		*In vivo* NMRS	[41]
Degree of saturation				
In vitro				
High abundance of unsaturated metabolites; decrease upon differentiation	ESC (human and mouse)	Neurons, cardiomyocytes (human and mouse)	MS of cell extracts	[44]
***Ex vivo* (rodent)**				
High degree of unsaturation of brain phospholipids during the first 3 postnatal weeks	P1, P7, P21 rat brain	Adult rat brain (5 months)	NMRS of tissue extracts	[39]
N-acetyl aspartate				
In vitro				
High abundance; twice the concentration found in neurons	O-2A progenitors (rat)	Neurons, astrocytes, oligodendrocytes, meningeal cells (rat)	NMRS of cell extracts	[13]
***Ex vivo* (rodent)**				
Increase with rat brain maturation during the first 4 postnatal weeks	P1–P28 rat brain	Adult rat brain	NMRS, HPLC of tissue extracts	[38]
Increase with rat brain maturation during the first 3 postnatal weeks	P1–P21 rat brain	Adult rat brain	NMRS of tissue extracts	[39]
***In vivo* (rodent)**				
Linear increase with rat brain maturation during the first 4 postnatal weeks	P1–P28 rat brain		*In vivo* NMRS	[37]
Increase with mouse brain maturation during the first 3 postnatal month	P10–P90 mouse brain		*In vivo* NMRS	[45]
***In vivo* (human)**				
Increase with maturation of human fetal brain during gestational weeks 30–41	Human fetal brain		*In vivo* NMRS	[40]
Increase with maturation of human fetal brain during gestational weeks 22–39	Human fetal brain		*In vivo* NMRS	[41]

Table 1 (*Continued*)

Findings	Stem cell types/tissues	Control cell types/tissues	Method	Refs
Amino acids				
In vitro				
High abundance, especially ala[c], glu, gly, asp	O-2A progenitors (rat)	Neurons, astrocytes, oligodendrocytes, meningeal cells (rat)	NMRS of cell extracts	[13]
High abundance, especially of ala, glu, gln, gly	O-2A progenitors (rat)	Neurons, astrocytes (rat)	NMRS of cell pellets (MAS[d])	[15]
Dependence of threonine catabolism	ESC (mouse)		MS of cell extracts	[50]
Positive correlation between clonogenicity and an NMR-spectral region dominated by signals of glu (2.28–2.38 ppm)	BTIC (human glioblastoma)		NMRS of cell suspensions	[14]
Release of ala, glu, gly, and ornithine (no consumption)	MSC (human)		HPLC of cell culture supernatants	[48]
***Ex vivo* (rodent)**				
Decrease of taurine with rat brain maturation during the first 3 postnatal weeks	P1–P21 rat brain	Adult rat brain	NMRS of tissue extracts	[39]
***In vivo* (rodent)**				
Increase of glu and gln, decrease of gly and ala between P10 and P20	P10–P90 mouse brain		*In vivo* NMRS	[45]
Presence of taurine at the first postnatal day; not detectable between P4 and P28	P1 rat brain	P4–P28	*In vivo* NMRS	[37]
Decrease of taurine during the first postnatal month	P10–P30 rat brain	P40–P90 rat brain	*In vivo* NMRS	[45]
Glucose/energy metabolism				
In vitro				
Preference for glycolysis	LT-HSC (mouse)	Bone marrow-derived lineages (mouse)	MS of cell extracts (lac[e]), OCR[f], ATP assay	[54]
Preference for glycolysis	ESC, iPSC (human)	Fibroblasts (human)	HPLC of cell extracts (ATP), OCR, lac release assay	[55]
Shift from oxidative phosphorylation to glycolysis during neuronal differentiation	ESC (mouse)	Neuronal-differentiated cells (mouse)	OCR, glc[g] uptake assay, lac release assay	[59]
Majority of ATP production through oxidative phosphorylation; shift to glycolysis during neural differentiation	ESC (human)	Neuronal-differentiated ESC, that is, NSC (human)	OCR, ATP demand assay	[60]
Preference for glycolysis	ESC, iPSC (human)	Keratinocytes, fibroblasts (human)	MS of cell extracts	[56]
Preference for oxidative phosphorylation	BTIC (human glioblastoma cell line U87)	Differentiated glioma cells	OCR, lac release assay, ATP assay, glc uptake assay	[58]
Preference for glycolysis	BTIC (human glioblastoma cell line U87, xenografted in mouse)	Glioblastoma cell line (human, U87)	OCR, lac release assay, ATP assay, glc uptake assay	[57]
High variation of glycogen after passaging	ESC (human)		RS	[67]
***In vivo* (rodent)**				
Linear increase of phosphocreatine during the first 4 postnatal weeks	P1–P28 rat brain		*In vivo* NMRS	[37]
Increase of creatine during the first 3 postnatal months	P10–P90 mouse brain		*In vivo* NMRS	[45]
Relative RNA abundance				
***In vitro* (rodent)**				
Decrease of RNA-to-protein ratio during differentiation	ESC (mouse)	Differentiated ESC (murine embryonic bodies)	RS	[64]
Decrease of RNA-to-protein ratio during glial differentiation	NSC (mouse)	NSC-derived glial cells	RMS	[66]
***In vitro* (human)**				
Decrease during cardiomyogenic differentiation	ESC (human)	ESC-derived cardiomyocytes, fetal cardiomyocytes	RS	[65]

[a]P28, postnatal day 28.

[b]HPLC, high-performance liquid chromatography.

[c]Standard three-letter code for amino acids.

[d]MAS, magic angle spinning (a.k.a. solid-state NMRS).

[e]lac, lactate.

[f]OCR, oxygen consumption rate.

[g]glc, glucose.

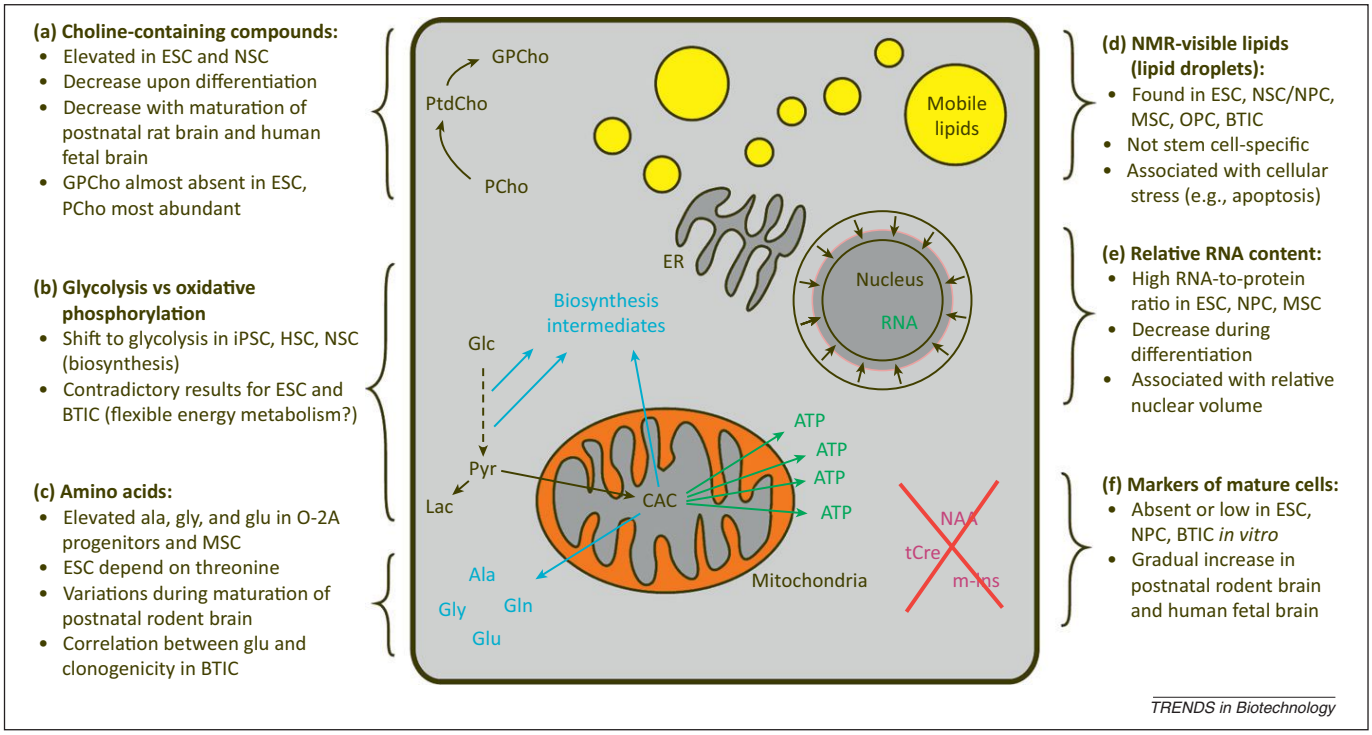

Figure 1. Major stem cell-specific findings of metabolic profiling approaches. **(a)** Choline-containing compounds decrease with differentiation and maturation. **(b)** Predominant energy metabolism via glycolysis in most but not all pluripotent cells. **(c)** Certain amino acids, for example, ala, gly, glu, and thr, are associated with stem cell metabolism. **(d)** Under stress conditions, many stem cell types show unspecific NMR-visible lipid droplets. **(e)** Relative RNA content and nuclear size decreases with differentiation. **(f)** Markers of mature cells, for example, NAA, tCre, and m-Ins are virtually absent in stem cells and appear/increase with maturation. Abbreviations: Ala, alanine; ATP, adenosine triphosphate; BTIC, brain tumor-initiating cell; CAC, citric acid cycle; ER, endoplasmic reticulum; ESC, embryonic stem cell; Glc, glucose; Gln, glutamine; Glu, glutamate; Gly, glycine; GPCho, glycerophosphocholine; HSC, hematopoietic stem cell; iPSC, induced pluripotent (stem) cell; Lac, lactate; m-Ins, myo-inositol; MSC, mesenchymal stem cell; NAA, N-acetyl aspartate; NPC, neural progenitor cell; NSC, neural stem cell; O-2A progenitor, oligodendrocyte-type-2 astrocyte progenitor cell; OPC, oligodendrocyte progenitor cell; PCho, phosphocholine; PtdCho, phosphatidylcholine; Pyr, pyruvate; tCre, creatine-containing compounds (creatine and phosphocreatine).

proliferation [35]. Especially in tumor cells, increased tCho is one of the major aberrant findings *in vitro* [36] and *in vivo* [17]. Although not yet possible in NMRS of intact cells or *in vivo*, resolving the different species of choline-containing compounds broadens the information content significantly. For instance, phosphocholine (PCho) mainly occurs during membrane synthesis as an intermediate of the major membrane lipid species phosphatidylcholine (PdtCho), whereas glycerophosphocholine (GPCho) is predominantly found upon membrane breakdown, that is, during degradation of PtdCho [35]. Therefore, the relative amount of PCho and GPCho yields information on synthesis or degradation, respectively, whereas tCho merely is a surrogate marker for 'membrane turnover' [17]. Nevertheless, the most consistent data associated with immaturity were found for tCho. In early postnatal rodent brains, it was shown that tCho decreases with maturation [37–39], and employing *in vivo* NMRS within the human fetal brain during gestation, a gradual decline of tCho could also be observed [40,41]. Interestingly, pluripotent ESCs *in vitro* were also characterized by marked tCho signals with respect to neuralized ESCs (NSCs) and other differentiated neural cell types, for example, neurons, astrocytes, and even when compared to various tumor cell lines [42,43]. Moreover, the application of high-resolution NMRS of cellular extracts was used to discriminate signals of free choline, PCho, and GPCho in these ESC studies. Surprisingly, GPCho was found to be absent in pluripotent ESCs, whereas PCho was most abundant [42]. Upon differentiation, however, the ratio of PCho to GPCho decreased in both ESCs and CSCs [43].

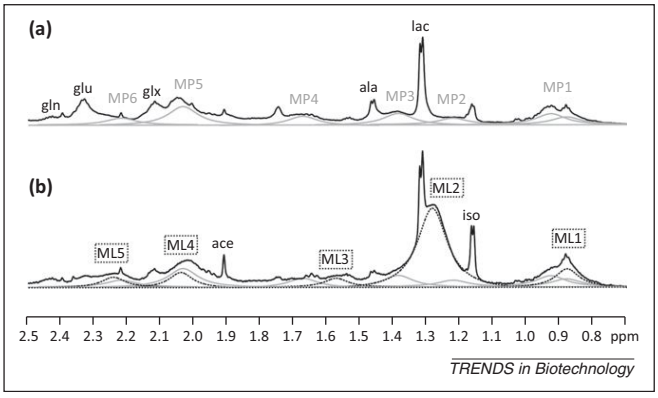

Figure 2. NMR spectra of intact neural progenitor cells (NPCs) with and without ML signals. **(a)** Five days after the last passage, that is, during the log phase of proliferation, no ML signals are observed. **(b)** Ten days after the last passage, that is, upon confluence, dominating ML signals are visible. Gray lorentzian curves: mobile protein (MP); dashed lorentzian curves: mobile lipid (ML). Abbreviations: gln, glutamine; glu, glutamate; glx, glutamine and/or glutamate; ace, acetate; ala, alanine; lac, lactate; iso, isopropanol. Approximately 5 million cells per sample. Adapted from [32].

Degree of saturation

The degree of saturation of phospholipid fatty acyl chains can also be associated with stem cells and cellular

maturation. In a recent study on early postnatal rat brains, a high degree of unsaturated brain phospholipids could be found within the organic phase of brain extracts [39]. Also, a high abundance of unsaturated metabolites was detected in ESCs [44]. Upon differentiation to neurons or cardiomyocytes, this tendency towards unsaturation vanished, and also in rat brains the relative amount of unsaturated phospholipids decreased with maturation [39]. The high degree of unsaturation of metabolites may allow stem cells to react to oxidative stimuli, for example, upon lesions or inflammation [44].

NAA and neuronal differentiation

In accordance with the decrease in tCho, the gradual increase of NAA both in early postnatal rodent brain [37–39,45] and *in utero* in human fetal brain during maturation [40,41] represents a second major consistent finding of metabolic profiling associated with stem cells. Since the very beginning of *in vivo* NMRS in human brains, NAA is considered 'the' marker of functional neurons, although the origin, function, and specificity of NAA remain elusive [16,17]. Interestingly, significant amounts of NAA could also be observed in oligodendrocyte-type-2 astrocyte (O-2A) progenitors [13,46,47], OPCs [46,47], and mature oligodendrocytes [47]. Another *in vivo* surrogate marker for functional neural cells, myo-inositol, was low in early postnatal mouse brains and doubled within the first three postnatal months [45]. Myo-inositol was previously described specifically for astrocytes *in vitro* and gliosis *in vivo* [16]. Interestingly, *in vitro*, myo-inositol was also found in NSCs but not ESC extracts [42] and in glioblastoma BTIC suspensions [14] pointing towards an early onset of a metabolic shift both during neurogenesis and tumorigenesis, respectively.

Amino acids

Amino acids are one major group of intermediates for biosynthesis and may play a key role in the discrimination of proliferative and metabolic states of stem cells and differentiated cells. Surprisingly, a consistent high amount of certain non-essential amino acids, such as alanine and glycine, has been found in highly diverse aspects of stemness, for example, *in vitro* in O-2A progenitors [13,15], in MSCs [48], and *in vivo* in early postnatal mouse brains [45]. Furthermore, in the developing brain of early postnatal rats and mice, a consistent presence of taurine could also be observed, which decreased upon maturation [37,39,45]. Very recently, taurine was shown to stimulate proliferation of NSCs and NPCs *in vitro* [49] suggesting an important role of taurine in early neural development. Additionally, a single amino acid, threonine, is crucial for murine ESC propagation *in vitro* [50,51]. For glioblastoma BTICs, we found a significant correlation between clonogenicity, that is, the *in vitro* hallmark of TICs, and the integral over an NMR spectral region dominated by glutamate signals (2.28–2.38 ppm) [14]. Unfortunately, to date, the relationship between these selective deviations in amino acid metabolism and any characteristics of stemness has not been understood.

Glycolysis and pluripotency

The synthesis of amino acids via glycolytic and citric acid cycle (CAC) intermediates is a key requirement for proliferation, because during cell division the complete cellular biomass needs to be doubled [52,53]. Very recently, a general preference for a glycolytic and anaplerotic metabolism of glucose at the expense of effective ATP production by means of complete oxidative phosphorylation could be shown for long-term HSCs (LT-HSCs) [54], ESCs and iPSCs [55,56], and even for glioma CSCs [57], although these findings were challenged simultaneously in the case of glioma CSCs [58] and ESCs [59,60]. Even more confusing, a shift from oxidative phosphorylation to glycolysis during neural differentiation was shown for human ESCs [60], mouse ESCs [59], and the developing mouse brain *in vivo* [59]. However, reprogramming of somatic cells to pluripotent stem cells requires a switch from oxidative metabolism to glycolysis [61]. Tightly related to the energy metabolism – especially within the brain – the concentration of creatine-containing compounds (tCre) was also shown to be dependent on the state of maturation. Gradually increasing tCre was found in postnatal rat and mouse brain upon developmental progression [37,45]. As the brain requires a highly sophisticated multicomponent network of fuel supply in order to satisfy its high demand for energy, for example, based on lactate shuttling from glial cells to neuronal axons followed by oxidative phosphorylation within neurons, the increase of secondary energy-buffering systems, like the phosphorylation of creatine during maturation, seems plausible.

Relative abundance of RNA and macromolecules

Most of the studies in which optical spectroscopy was applied to investigate stem cells concluded that during differentiation the relative amount of RNA decreases whereas the protein and lipid content increases (comprehensively reviewed in [62,63]). First shown for murine ESCs when differentiating to embryonic bodies [64], this decrease in RNA-to-protein ratio could also be observed in human ESCs during cardiomyogenesis [65] and in murine NSCs during differentiation to glial cells [66]. Subcellular RMS revealed a much higher RNA and DNA content within the nuclei of human MSCs and ESCs than within the cytoplasm and vice versa for proteins and lipids [19,63]. Therefore, it was concluded that the relative size of the nucleus and the cytoplasm gave rise to the relative drop in RNA signals upon differentiation, that is, a shrinkage of the nucleus accompanied by an increasing cytoplasmic volume [19]. Apart from RNA, RS could also be used to determine the glycogen content of human ESCs [67,68], which revealed a significant sample-to-sample variation of glycogen within the first 72 h after passaging [67]. Furthermore, optical spectroscopy also proved beneficial in monitoring osteogenesis by means of mineralization-specific signals [63].

A matter of methodology

As mentioned earlier, the reviewed findings on putative stem cell-specific metabolic profiles must be considered in the light of differences in cellular statuses, experimental setups, and profiling approaches. The metabolome of stem

cells *in vivo*, for example, in the developing brain or within hypoxic niches of an adult organism, is assumed to hardly match that of *in vitro* propagated stem/progenitor cells. For instance, cultured tumor cells were reported to preferentially metabolize glucose via aerobic glycolysis – the Warburg effect [52]; however, recent *in vivo* studies employing ^{13}C-NMRS revealed a more complex metabolism of isotopically labeled glucose in brain tumors including anaplerosis and oxidative phosphorylation [69,70]. Therefore, in the case of cultured stem cells, results pointing at, for example, a preference for glycolysis [54–57] or oxidative phosphorylation [58,60] should be considered as what they are: snapshots of a cellular status profoundly adapted to an artificial environment. Furthermore, the profiling approach itself co-determines the anticipated outcome. The comparison of (i) metabolic footprints, that is, small molecules taken up or released from/to the cell culture medium; (ii) metabolic fingerprints, that is, intracellular metabolites extracted by means of cell lysis; and (iii) intracellular pools of mobile molecules and flexible macromolecular motifs within intact cells, either *in vitro* as cell suspensions or *in vivo* within the crosstalking cellular network, is far from straightforward. Both MS and NMRS show high resolution, sensitivity, and throughput when applied to extracted or dissolved molecules [9,10]. However, both the temporal dimension, that is, the possibility of monitoring metabolism, and the information on intracellular molecular mobility is lost as a consequence of sample preparation (cell extraction). Here, optical spectroscopy, especially RMS, offers the unmatched advantage of yielding metabolome features on a single-cell level. However, to date, the main informative value of RMS on stem cells is the decrease of RNA-to-protein ratio as a consequence of nuclear shrinkage.

In vivo approaches by means of localized NMRS at MRI scanners provide insight into the physiologic metabolism of cells – in some cases, predominantly stem/progenitor cells, for example, in the developing brain [40,41,45]. However, the intrinsic low resolution and sensitivity of NMRS *in vivo* – especially at clinical scanners – reduces the informational content to a few metabolites [17]. In our opinion, the gap between snapshot *in vitro* approaches and low-resolution clinical molecular imaging could be filled by NMRS of intact cells perfused with defined medium inside the NMR spectrometer during the measurement. This perfusion approach would enable (i) adaptable and controlled cell culture conditions; (ii) metabolic monitoring for days with a temporal resolution of only a few minutes; (iii) enrichment of cells-of-interest, for example, regarding stem cell potential, differentiation state, lineage commitment, and proliferation status; (iv) tracing various metabolic responses to external stimuli, for example, growth factors or putative drugs; and (v) spying on metabolic crosstalk of different cell types, that is, stem cells and immune cells.

Concluding remarks

Tentatively, the findings reviewed above can be grouped in two categories: (i) *in vivo* data of human fetal brain and rodent early postnatal brain, and (ii) *in vitro* results on cultured cells at different stages of maturation. These stages range from pluripotent embryonic or induced stem cells, to multipotent stem/progenitor cells restricted to the neural, hematopoietic, or mesenchymal lineage, to oligopotent/unipotent precursors of the glial lineage (O-2A progenitors and OPCs), to the pathophysiologic counterparts, that is, CSCs or TICs. *In vivo* studies consistently reveal a gradual assimilation towards the adult metabolic profile along maturation, with increases of markers of functionality/maturity (NAA, creatine, and myo-inositol) and decreases of metabolites associated with proliferation and biomass synthesis (amino acids, choline, and taurine). Unfortunately, apart from consistently high RNA-to-protein ratios as revealed by optical spectroscopy, especially RS, findings on cultured stem/progenitor cells cannot be easily generalized. This is most probably due to the heterogeneity of investigated cell types, stem cell states, culture conditions, and applied methods. At least on the metabolic level, cell culture hardly resembles the *in vivo* condition for stem cells, for example, regarding oxygenation, availability of nutrients, crosstalk to other cell types, growth factors, etc. Therefore, findings on metabolic profiling approaches on cultured stem cells still have to prove their value. In future studies on stem cell metabolomes *in vitro*, culture conditions should adhere as closely as possible to *in vivo* physiological conditions.

Acknowledgments

This work has been made possible through the support from the State Government of Salzburg (Austria), the Bavarian State Ministry of Sciences, Research, and Arts (ForNeuroCell2), the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF 01GN0978), the Propter Homines Foundation, the Fonds zur Förderung der wissenschaftlichen Forschung (FWF), Austria Special Research Program (SFB) F44 'Cell Signaling in Chronic CNS Disorders', the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. HEALTH-F2-2011-278850 (INMiND) and no. HEALTH-F2-2011-279288 (IDEA).

References

1 Sandner, B. *et al.* (2012) Neural stem cells for spinal cord repair. *Cell Tissue Res.* 349, 349–362
2 Doetsch, F. *et al.* (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97, 703–716
3 Encinas, J.M. *et al.* (2011) Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. *Cell Stem Cell* 8, 566–579
4 Steffenhagen, C. *et al.* (2011) Identity, fate and potential of cells grown as neurospheres: species matters. *Stem Cell Rev.* 7, 815–835
5 Paul, G. *et al.* (2012) The adult human brain harbors multipotent perivascular mesenchymal stem cells. *PLoS ONE* 7, e35577
6 Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676
7 Singh, S.K. *et al.* (2004) Cancer stem cells in nervous system tumors. *Oncogene* 23, 7267–7273
8 Beier, C.P. and Beier, D. (2011) CD133 negative cancer stem cells in glioblastoma. *Front. Biosci. (Elite Ed.)* 3, 701–710
9 Dunn, W.B. *et al.* (2011) Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem. Soc. Rev.* 40, 387–426
10 Khoo, S.H.G. and Al-Rubeai, M. (2007) Metabolomics as a complementary tool in cell culture. *Biotechnol. Appl. Biochem.* 47, 71–84
11 Jain, M. *et al.* (2012) Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* 336, 1040–1044
12 Kell, D.B. *et al.* (2005) Metabolic footprinting and systems biology: the medium is the message. *Nat. Rev. Microbiol.* 3, 557–565

13 Urenjak, J. *et al.* (1993) Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J. Neurosci.* 13, 981–989
14 Ramm, P. *et al.* (2011) 1H-NMR spectroscopy of glioblastoma cancer stem cells. *Stem Cells Dev.* 20, 2189–2195
15 Griffin, J.L. *et al.* (2002) Spectral profiles of cultured neuronal and glial cells derived from HRMAS (1)H NMR spectroscopy. *NMR Biomed.* 15, 375–384
16 Duarte, J.M.N. *et al.* (2012) The neurochemical profile quantified by *in vivo* 1H NMR spectroscopy. *Neuroimage* 61, 342–362
17 Mountford, C.E. *et al.* (2010) Neurospectroscopy: the past, present and future. *Chem. Rev.* 110, 3060–3086
18 Rubakhin, S.S. *et al.* (2011) Profiling metabolites and peptides in single cells. *Nat. Methods* 8, S20–S29
19 Downes, A. *et al.* (2011) Raman spectroscopy and CARS microscopy of stem cells and their derivatives. *J. Raman Spectrosc.* 42, 1864–1870
20 Gronwald, W. *et al.* (2011) Detection of autosomal dominant polycystic kidney disease by NMR spectroscopic fingerprinting of urine. *Kidney Int.* 79, 1244–1253
21 Blankenberg, F.G. *et al.* (1996) Detection of apoptotic cell death by proton nuclear magnetic resonance spectroscopy. *Blood* 87, 1951–1956
22 Hakumäki, J.M. and Kauppinen, R.A. (2000) 1H NMR visible lipids in the life and death of cells. *Trends Biochem. Sci.* 25, 357–362
23 Delikatny, E.J. *et al.* (2011) MR-visible lipids and the tumor microenvironment. *NMR Biomed.* 24, 592–611
24 Zoula, S. *et al.* (2003) Correlation between the occurrence of 1H-MRS lipid signal, necrosis and lipid droplets during C6 rat glioma development. *NMR Biomed.* 16, 199–212
25 Barba, I. *et al.* (1999) The relationship between nuclear magnetic resonance-visible lipids, lipid droplets, and cell proliferation in cultured C6 cells. *Cancer Res.* 59, 1861–1868
26 Quintero, M.R. *et al.* (2007) A possible cellular explanation for the NMR-visible mobile lipid (ML) changes in cultured C6 glioma cells with growth. *Biochim. Biophys. Acta* 1771, 31–44
27 Manganas, L.N. *et al.* (2007) Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. *Science* 318, 980–985
28 Hoch, J.C. *et al.* (2008) Comment on 'magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain'. *Science* 321, 640, author reply 640
29 Friedman, S.D. (2008) Comment on 'Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain'. *Science* 321, 640, author reply 640
30 Jansen, J.F.A. *et al.* (2008) Comment on 'Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain'. *Science* 321, 640, author reply 640
31 Djurić, P.M. *et al.* (2008) Response to Comments on 'Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain'. *Science* 321, 640
32 Ramm, P. *et al.* (2009) A nuclear magnetic resonance biomarker for neural progenitor cells: is it all neurogenesis? *Stem Cells* 27, 420–423
33 Loewenbrück, K.F. *et al.* (2011) Proton MR spectroscopy of neural stem cells: does the proton-NMR peak at 1.28 ppm function as a biomarker for cell type or state? *Rejuvenation Res.* 14, 371–381
34 Biebl, M. *et al.* (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci. Lett.* 291, 17–20
35 Podo, F. (1999) Tumour phospholipid metabolism. *NMR Biomed.* 12, 413–439
36 Glunde, K. *et al.* (2011) Choline metabolism in malignant transformation. *Nat. Rev. Cancer* 11, 835–848
37 Hida, K. *et al.* (1992) *In vivo* 1H and 31P NMR spectroscopy of the developing rat brain. *Magn. Reson. Med.* 23, 31–36
38 Florian, C.L. *et al.* (1996) Regional and developmental variations in metabolite concentration in the rat brain and eye: a study using 1H NMR spectroscopy and high performance liquid chromatography. *Neurochem. Res.* 21, 1065–1074
39 Zancanaro, C. *et al.* (2001) NMR spectroscopic analysis of rat brain development: *in vitro* proton and carbon studies of whole tissue and its phospholipid fraction. *Dev. Neurosci.* 23, 107–112
40 Kok, R.D. *et al.* (2002) Maturation of the human fetal brain as observed by 1H MR spectroscopy. *Magn. Reson. Med.* 48, 611–616
41 Girard, N. *et al.* (2006) Assessment of normal fetal brain maturation in utero by proton magnetic resonance spectroscopy. *Magn. Reson. Med.* 56, 768–775
42 Jansen, J.F.A. *et al.* (2006) Stem cell profiling by nuclear magnetic resonance spectroscopy. *Magn. Reson. Med.* 56, 666–670
43 Romanska, H.M. *et al.* (2009) Nuclear magnetic resonance detects phosphoinositide 3-kinase/Akt-independent traits common to pluripotent murine embryonic stem cells and their malignant counterparts. *Neoplasia* 11, 1301–1308
44 Yanes, O. *et al.* (2010) Metabolic oxidation regulates embryonic stem cell differentiation. *Nat. Chem. Biol.* 6, 411–417
45 Kulak, A. *et al.* (2010) Neurochemical profile of the developing mouse cortex determined by *in vivo* 1H NMR spectroscopy at 14.1 T and the effect of recurrent anaesthesia. *J. Neurochem.* 115, 1466–1477
46 Urenjak, J. *et al.* (1992) Specific expression of N-acetylaspartate in neurons, oligodendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes *in vitro*. *J. Neurochem.* 59, 55–61
47 Bhakoo, K.K. and Pearce, D. (2000) *In vitro* expression of N-acetyl aspartate by oligodendrocytes: implications for proton magnetic resonance spectroscopy signal *in vivo*. *J. Neurochem.* 74, 254–262
48 Higuera, G.A. *et al.* (2012) Patterns of amino acid metabolism by proliferating human mesenchymal stem cells. *Tissue Eng. Part A* 18, 654–664
49 Hernández-Benítez, R. *et al.* (2012) Taurine stimulates proliferation and promotes neurogenesis of mouse adult cultured neural stem/progenitor cells. *Stem Cell Res.* 9, 24–34
50 Wang, J. *et al.* (2009) Dependence of mouse embryonic stem cells on threonine catabolism. *Science* 325, 435–439
51 Wang, J. *et al.* (2011) Metabolic specialization of mouse embryonic stem cells. *Cold Spring Harb. Symp. Quant. Biol.* 76, 183–193
52 Vander Heiden, M.G. *et al.* (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033
53 Lunt, S.Y. and Vander Heiden, M.G. (2011) Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu. Rev. Cell Dev. Biol.* 27, 441–464
54 Simsek, T. *et al.* (2010) The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. *Cell Stem Cell* 7, 380–390
55 Varum, S. *et al.* (2011) Energy metabolism in human pluripotent stem cells and their differentiated counterparts. *PLoS ONE* 6, e20914
56 Panopoulos, A.D. *et al.* (2012) The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. *Cell Res.* 22, 168–177
57 Zhou, Y. *et al.* (2011) Metabolic alterations in highly tumorigenic glioblastoma cells: preference for hypoxia and high dependency on glycolysis. *J. Biol. Chem.* 286, 32843–32853
58 Vlashi, E. *et al.* (2011) Metabolic state of glioma stem cells and nontumorigenic cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16062–16067
59 Fornazari, M. *et al.* (2011) Neuronal differentiation involves a shift from glucose oxidation to fermentation. *J. Bioenerg. Biomembr.* 43, 531–539
60 Birket, M.J. *et al.* (2011) A reduction in ATP demand and mitochondrial activity with neural differentiation of human embryonic stem cells. *J. Cell Sci.* 124, 348–358
61 Folmes, C.D.L. *et al.* (2012) Energy metabolism plasticity enables stemness programs. *Ann. N. Y. Acad. Sci.* 1254, 82–89
62 Chan, J.W. and Lieu, D.K. (2009) Label-free biochemical characterization of stem cells using vibrational spectroscopy. *J. Biophotonics* 2, 656–668
63 Downes, A. *et al.* (2010) Optical spectroscopy for noninvasive monitoring of stem cell differentiation. *J. Biomed. Biotechnol.* 2010, 101864
64 Notingher, I. *et al.* (2004) *In situ* spectroscopic study of nucleic acids in differentiating embryonic stem cells. *Vib. Spectrosc.* 35, 199–203
65 Chan, J.W. *et al.* (2009) Label-free separation of human embryonic stem cells and their cardiac derivatives using Raman spectroscopy. *Anal. Chem.* 81, 1324–1331
66 Ghita, A. *et al.* (2012) Cytoplasmic RNA in undifferentiated neural stem cells: a potential label-free Raman spectral marker for assessing the undifferentiated status. *Anal. Chem.* 84, 3155–3162
67 Konorov, S.O. *et al.* (2011) Evidence of marked glycogen variations in the characteristic Raman signatures of human embryonic stem cells. *J. Raman Spectrosc.* 42, 1135–1141

68 Konorov, S.O. *et al.* (2011) Absolute quantification of intracellular glycogen content in human embryonic stem cells with Raman microspectroscopy. *Anal. Chem.* 83, 6254–6258
69 Marin-Valencia, I. *et al.* (2012) Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain *in vivo*. *Cell Metab.* 15, 827–837
70 Maher, E.A. *et al.* (2012) Metabolism of [U-(13) C]glucose in human brain tumors *in vivo*. *NMR Biomed.* 25, 1234–1244
71 Dunnett, S.B. and Rosser, A.E. (2011) Clinical translation of cell transplantation in the brain. *Curr. Opin. Organ Transplant.* 16, 632–639
72 Piccini, P. *et al.* (1999) Dopamine release from nigral transplants visualized *in vivo* in a Parkinson's patient. *Nat. Neurosci.* 2, 1137–1140
73 Freed, C.R. *et al.* (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* 344, 710–719
74 Olanow, C.W. *et al.* (2003) A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* 54, 403–414
75 Van Laake, L.W. *et al.* (2007) Monitoring of cell therapy and assessment of cardiac function using magnetic resonance imaging in a mouse model of myocardial infarction. *Nat. Protoc.* 2, 2551–2567
76 Chen, L.W. *et al.* (2011) Potential application of induced pluripotent stem cells in cell replacement therapy for Parkinson's disease. *CNS Neurol. Disord. Drug Targets* 10, 449–458
77 Brazzini, A. *et al.* (2010) Intraarterial autologous implantation of adult stem cells for patients with Parkinson disease. *J. Vasc. Interv. Radiol.* 21, 443–451
78 O'Brien, C.A. *et al.* (2010) Cancer stem cells and self-renewal. *Clin. Cancer Res.* 16, 3113–3120
79 Zhou, B-B.S. *et al.* (2009) Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. *Nat. Rev. Drug Discov.* 8, 806–823
80 Morgan, R.A. *et al.* (2012) Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. *Hum. Gene Ther.* 23, 1043–1053
81 Florio, T. and Barbieri, F. (2012) The status of the art of human malignant glioma management: the promising role of targeting tumor-initiating cells. *Drug Discov. Today* 17, 1103–1110
82 Carro, M.S. *et al.* (2010) The transcriptional network for mesenchymal transformation of brain tumours. *Nature* 463, 318–325
83 Dang, C.V. and Semenza, G.L. (1999) Oncogenic alterations of metabolism. *Trends Biochem. Sci.* 24, 68–72
84 Menon, L.G. *et al.* (2012) Imaging of human mesenchymal stromal cells: homing to human brain tumors. *J. Neurooncol.* 107, 257–267
85 Roger, M. *et al.* (2011) The potential of combinations of drug-loaded nanoparticle systems and adult stem cells for glioma therapy. *Biomaterials* 32, 2106–2116
86 Batrakova, E.V. *et al.* (2011) Cell-mediated drug delivery. *Expert Opin. Drug Deliv.* 8, 415–433
87 Tabatabai, G. *et al.* (2005) Lessons from the bone marrow: how malignant glioma cells attract adult haematopoietic progenitor cells. *Brain* 128, 2200–2211
88 Tabatabai, G. *et al.* (2011) Stem cell-mediated gene therapies for malignant gliomas: a promising targeted therapeutic approach? *Discov. Med.* 11, 529–536
89 Dhermain, F.G. *et al.* (2010) Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. *Lancet Neurol.* 9, 906–920