Reprogramming and Carcinogenesis-Parallels and Distinctions

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Invited contribution

Reprogramming and carcinogenesis - parallels and distinctions

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Abbreviations: CDK - Cyclin-Dependent Kinase; CSC - cancer stem cells; DSB - double strand breaks ; ECM - extracellular matrix; ES cell – embryonic stem cell; GFP – green fluorescent protein; GSK3 - glycogen synthase kinase-3; HN - Hemagglutinin-neuraminidase; iPS – induced pluripotent stem cells; MSFs - myocardial scar fibroblasts; RB - Retinoblastoma protein; RGD - arginine-glycine-aspartic acid; RISC – RNA-induced silencing complex; Shc - Src homology 2 domain-containing.

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Abstract

Rapid progress made in various areas of regenerative medicine in recent years, occurred both at the cellular level, with the Nobel prize-wining discovery of reprogramming (generation of induced-pluripotent stem cells (iPS)), and also at the biomaterial level. The use of four transcription factors Oct3/4, Sox2, c-Myc, and Klf4 (called commonly 'Yamanaka factors') for the conversion of differentiated cells, back to the pluripotent/embryonic stage has opened virtually endless, and ethically acceptable source of stem cells for medical use. Various types of stem cells are becoming increasingly popular as starting components for the development of replacement tissues, or artificial organs. Interestingly, many of the transcription factors key to the maintenance of stemness phenotype in various cells, are also over-expressed in cancer (stem) cells, and some of them may find the use as prognostic factors. In this review, we give brief introduction to various methods of iPS creation, followed by overview of factors known to interfere with the efficiency of reprogramming. Next, we discuss similarities between cancer stem cells and various stem cell types. Final paragraphs are dedicated to interaction of biomaterials with tissues, various adverse reactions generated as results of such interactions, and measures available, that allow for mitigation of such negative effects.

Key words: bioglass; p53, senescence, Yamanaka factor; trans-differentiation,

1. Introduction

Reprogramming, or reversing adult (partially) differentiated cell into a cell with properties very closely resembling embryonic stem (ES) cells, called 'induced-pluripotent stem' (iPS) cells, by the expression of a set of transcription factors, has been first achieved in 2006 (Takahashi and Yamanaka, 2006). Initially, the induction of pluripotent stem cells was carried out by introduction of four factors, Oct3/4, Sox2, c-Myc, and Klf4 (called commonly 'Yamanaka factors') to murine (embryonic) fibroblasts, under ES cell culture conditions. Nowadays, protocols exists, where only two or three factors are used, with c-Myc appearing to only enhance the process rather than being indispensible. For example, Li and colleagues reprogrammed the cells without introducing Sox2 transcription factor, showing that only Oct4 and Klf4 and glycogen synthase kinase-3 (GSK3) -inhibitor CHIR 99021 are sufficient for murine embryonic fibroblasts reprogramming. They indicate that GSK3-inhibitor can replace the reprogramming-related effects of Sox2 transcription factor (Banito et al., 2009).

While initial reprogramming procedures were performed in murine embryonic- or adult fibroblasts, it is becoming increasingly evident that (*i*) "younger" cells are reprogrammed with higher efficiency, (*ii*) cells that by their nature already express some of the reprogramming factors become reprogrammed with higher frequency and by using only some of the Yamanaka factors (Kim et al., 2009b), and (*iii*) cells expression lower amounts of p53 are also reprogrammed with higher efficiency. The later observation indicates that preprogramming itself induces enormous cellular stress, and thus it is a powerful inducer of p53-activated cell death, or senescence triggered by p53, $p16^{INK4a}$, and $p21^{CIP1}$ (Banito et al., 2009; Menendez et al., 2010). Thus, modern reprogramming protocols typically include apoptosis- and/or senescence inhibitors in the reprogramming medium.

2. Brief Overview of Various Reprogramming Methods

Before transcription-factor based reprogramming protocol had been published by Yamanaka group, such process could only be achieved either by transfer of nuclei of i.e. mature, differentiated cells into oocytes from which pronuclei were removed, or by fusion with ES cells (Moawad et al., 2011). Somatic cell nuclear transfer has sometimes been used in humans, but it is controversial because of ethical issues related to human egg destruction (Wilmut et al., 1997).

One of the most efficient methods to generate iPS cells is lentiviral transduction of Yamanaka factors (Nakagawa et al., 2008). In this approach retroviruses integrate into host's genome and allow for sustained expression of reprogramming factors at a sufficient level. Interestingly, newly generated iPS cells are able to methylate and switch-off the expression of lentivirally-introduced Yamanaka factors, while relying instead on the expression of the same intrinsic transcription factors (Hotta and Ellis, 2008). Lentiviruses have been used for the delivery of Yamanaka factors in a monocistronic form (one factor per virus), or as a multi-factor cassette (polycistronic, offered i.e. by Millipore) in a single lentiviral particle (Sommer et al., 2009). Lentiviral delivery of Yamanaka factors, like other retroviral methods, carry significant risk of insertion-mutagenesis related to their genomic integration (Hockemeyer et al., 2008; Sommer et al., 2009), therefore while they are a valuable research tools, they cannot be safely used in the clinic.

Contrary to report by Hotta and Ellis (Hotta and Ellis, 2008), viral transgenes may not always be inactivated completely, and during the longer culture period they may reactivate. It has been shown that chimeric mice produced from viral iPS cells frequently developed tumors, because of reactivation of c-Myc expressing virus, used as one of the original Yamanaka factors (Nakagawa et al., 2008). Some Yamanaka factors are also frequently upregulated by certain tumors and their expression level generally correlates with increased

metastatic potential and thus with bad prognosis (please see below) (Huang et al., 2011; Lengerke et al., 2011).

Adenoviral vectors are another option for the delivery of reprogramming factors. They do not integrate into host's genome, and some adenoviral vectors deliver genes with high efficiency to certain cell types like i.e. hepatocytes. In general however, lower efficiency and shorter expression kinetics, that would require repeated transductions to maintaining an adequate level of transgene expression makes adenoviral vectors less suitable for the task (Esteban et al., 2010; Sommer et al., 2009).

Reprogramming has been successfully achieved by Fusaki and colleagues using Sendai virus-based delivery of Yamanaka factors (non-integrating RNA-virus) (Fusaki et al., 2009). As the used Sendai virus-based vector was replication-deficient, its copies became diluted during cell divisions, and eventually virus-free iPS-cells were available. Remaining Sendai virus-harboring cells could be removed using antibody recognizing HN-surface marker (expressed only on Sendai virus-infected cells). Thus, this reprogramming-technique works without changes to genome.

Precisely due to the above highlighted safety issues, labs around the world aim at developing efficient non-viral reprogramming methods. In this way, one could obtain iPS cells without introducing changes into their genome (beside epigenetic changes). Such methods include (*i*) minicircle vectors, (*ii*) PiggyBac transposon system, (*iii*) already mentioned episomal vectors, and (*iv*) the delivery of Yamanaka factors as proteins. The common denominator of such methods is much lower reprogramming efficiency as compared to lentiviral vectors-based reprogramming.

Minicircle vectors-based reprogramming was for the first time described in 2010 (Jia et al., 2010). Minicircle vectors are cicular, nonviral DNA elements that are generated by PhiC31 integrase-based intramolecular recombination from parental plasmids that contain

sequences of interest (i.e. Oct3/4, Sox2, Nanog, Lin28, GFP). Expression of minicircle-coded genes lasts weeks, and occurs in both dividing- and nondividing cells. Minicircle vectors get introduced (transfected) into cells with higher efficiency, and typically yield higher expression levels of desired proteins, as they are less likely to be inactivated by cellular mechanisms targeting foreign nucleic acids. The used by Jia and colleagues plasmid contains a single cassette of four reprogramming factors and GFP as a marker sequences, all separated by self-cleavage peptide 2A sequences (Jia et al., 2010).

PiggyBac transposon reprogramming was first proposed by Kaji and colleagues in 2009 (Kaji et al., 2009). In this method transgene-sequence can be removed from integration site without changing host's DNA. The PiggyBac transposon carries a cassette that contains all four reprogramming factors, linked with 2A self-cleavage peptides. For removal purposes, the cassette containing reprogramming factors is flanked by *lox*P sites (Kaji et al., 2009). To remove the exogenous reprogramming factors, iPS-cells were transiently transfected with Cre. This system minimizes genome modification in iPS cells and enables complete elimination of nucleic acid sequences encoding exogenous reprogramming factors.

High-efficiency, synthetic mRNA-based reprogramming was recently described (Warren et al., 2010). To achieve that, authors used large quantities of synthetic mRNAs coding for Yamanaka factors, modified to overcome innate antiviral responses. Since mRNA is translated to protein in the cytoplasm they do not have to enter the nucleus, thus further minimizing chance of unwanted modifications of hosts DNA. This method appears to work fast and efficiently (reprogramming in just two-week period, much shorter than lentiviral-vector based reprogramming, and 4% efficiency – several orders of magnitude higher than lentiviral-vector based reprogramming) (Warren et al., 2010). The major drawback is the need to produce large quantities of high-quality, very long stretches of synthetic mRNA (rather technically challenging and expensive). Furthermore, the cellular RISC system

attempts to degrade such synthetic mRNAs (mechanism of RNA destruction like in RNA-interference technology) (van den Berg et al., 2008). To overcome that problem, researchers tested synthetic mRNAs chemically modified so that were not readily recognized as a foreign RNA. As an alternative to chemical synthesis, *in vitro* transcription systems exist, which allow for the synthesis of desired mRNAs.

Protein-based reprogramming carries the advantage that it does not cause any genetic changes. As already mentioned, current methods of protein-based reprogramming are several order of magnitudes less inefficient that lentiviral delivery of Yamanaka factors (Bass et al., 2009; Esteban et al., 2010). Typically, synthesized in bacteria Yamanaka factors are modified so that they express basic amino acids- or other transport peptides enabling to cross the cell membrane (Hauff et al., 2005). Table I summarizes current reprogramming methods highlighting their most important advantages and dissadvantages.

Beside reprogramming, and subsequent differentiation into desired cell type, several authors have recently reported the possibility of trans-differentiation, or conversion of one cell type to another one, while bypassing the iPS-state. Here are some examples. Recently, conversion of pancreatic cells to hepatocytes by the treatment with dexamethasone was reported (Eberhard and Jockusch, 2010). Another group converted B cells into hematopoetic multipotent progenitors and then reprogrammed them in to T-cells and macrophages (Cobaleda, 2010). Vierbuchen and colleagues describes direct conversion of fibroblasts into neurons by the expression of neural-lineage-specific transcription factors Ascl1, Brn2/Pou3f2 and Myt1l (Vierbuchen et al., 2010). Direct conversion of myocardial scar fibroblasts (MSFs) to cardiomyocytes, by infection of human MSFs with a lentivirus vector encoding the potent cardiogenic transcription factor myocardin was achieved (van Tuyn et al., 2007). Those are just a few examples of the strong scientific interest into direct conversion of one cell type into another.

3. Reprogramming, Antiproliferative Response, Apoptosis and Senescence

An activation of the antiproliferative response during reprogramming has been shown by several authors (Banito et al., 2009). The role of tumor suppressors in reprogramming is inherent at different levels and highlights how stressful the reprogramming is for the cell (Marion et al., 2009). p53 converts various upstream cellular-stress signals into downstream responses including cell cycle arrest, senescence, DNA repair and apoptosis (Vincent and Los, 2011). Several groups analyzed whether the expression of the reprogramming factors is sufficient to trigger directly an antiproliferative response and showed that the expression of the four Yamanaka factors, or combinations of Oct4, Sox2 and Klf4, in human or murine fibroblasts induces p53 and $p21^{CIP1}$ (Banito and Gil, 2010; Hong et al., 2009; Kawamura et al., 2009). Moreover, the same studies also suggest that a combination of Oct4 and Sox2 or even the individual factors is also sufficient to trigger the activation of p53 and/or $p21^{CIP1}$ (Banito et al., 2009; Kawamura et al., 2009). The main determinants of p53 activation during reprogramming have not been clearly established (Fig. 1). The expression of the four Yamanaka factors in human fibroblasts results oxidative stress and subsequent DNA damage, what leads to the activation of p53 (Egler et al., 2005; Vafa et al., 2002). In addition to p53, transcriptional profiling revealed that reprogramming also activates other genes involved in DNA replication and cell cycle progression such as: POLI, RCF4, MCM5, CCND1, CCND and the Cyclin-Dependent Kinase (CDK) inhibitors $p21^{CIP1}$ and $p16^{INK4a}$ (Banito et al., 2009; Mikkelsen et al., 2008; Sridharan et al., 2009).

3.1. Apoptosis and senescence as outcomes of aborted reprogramming

Murine and human fibroblasts expressing reprogramming factors suffer a cell-cycle arrest that presents multiple characteristics of senescence, such as upregulation of $p16^{INK4a}$ (Banito

et al., 2009). Upregulation of both BAX and its antagonist molecule BCL2 in response to the expression of Oct4, Sox2 and Klf4 enhance reprogramming (Kawamura et al., 2009). Other reports suggest that the expression of the reprogramming factors synergizes with the induction of DNA damage to trigger apoptosis. In such a scenario, the expression of BCL2 restores the ability of these cells to be reprogrammed (Marion et al., 2009). Therefore reprogramming is limited by both antiproliferative responses as happens during tumor suppression, in which both senescence and apoptosis are implicated.

3.2. Tumor suppressor loss, differentiation and oncogenic tranformation

Antiproliferative response is activated during the reprogramming process. However, this response is an obstacle in the development of pluripotent cell. Suppression of antiproliferative response increases the efficiency of reprogramming to a more dedifferentiated state but at the same time increases the oncogenic potential of cells. Genomic alterations that occur during reprogramming can cause inefficient antiproliferative response, enhance reprogramming and increase susceptibility for oncogenic transformation. Commitment of the cell depends on these complex relations (Fig. 1).

Several groups have shown that knocking down p53 in human or murine cells significantly increase the efficiency of reprogramming (Banito and Gil, 2010; Hong et al., 2009; Kawamura et al., 2009; Marion et al., 2009). The expression of MDM2 or a dominant-negative mutant of p53 also results in enhanced reprogramming, whereas the activation of p53 through different strategies reduced the reprogramming efficiency (Kawamura et al., 2009; Marion et al., 2009), emphasizing the importance of controlling p53 activity to modulate reprogramming. Similarly, low levels or absence of $p16^{INK4a}$ or $p21^{CIP1}$ expression leads to more efficient and faster reprogramming in mouse and human cells (Banito et al., 2009; Li et al., 2009).

Genomic instability that occurs during and after reprogramming (i.e. prolonged passaging of iPS cells) may contribute to the increase of transformation potential of iPS cells (Gore et al., 2011). Mutations in the p53 and $p16^{INK4a}$/Retinoblastoma protein (RB) pathways are found in the majority of tumors, thus any changes that would destabilize those pathways in iPS cells are particularly dangerous, as they would increase the probability of the emergence of iPS-derived tumors.

Defects in the p53 or the $p16^{INK4a}$/RB pathways have an impact on tumorigenesis as they appear to be especially common in tumors showing plasticity and loss of differentiation characteristics, thus increasing the pool of cancer stem cells (CSC) (Banito et al., 2009). p53 loss was associated with poorly differentiated thyroid cancers (Fagin et al., 1993), breast cancer (de Cremoux et al., 1999; Kochhar et al., 2005; Miller et al., 2005; Mizuno et al., 2010) lung cancer (Junttila et al., 2010), hematopoetic system malignacies (Chylicki et al., 2000; Feinstein et al., 1992), collateral mutations of p53 and PTEN are the most common tumor suppressor aberrations in glioblastomas, which are poorly differentiated, developmentally plastic brain tumors derived from the neuronal stem/progenitor cells (Zheng et al., 2008). Deletion of the three RB family proteins triggers the reprogramming of MEFs to generate CSC-like cells (Liu et al., 2009).

4. Reprogramming and Autophagy

4.1. Autophagy

Autophagy is a catabolic process that is initiated in cells which is facing stress or starvation conditions (Abounit et al., 2012) and, beside apoptosis and necrosis, if hyper-activated, it may constitute a separate cell death mechanism, removing damaged cells (Ghavami et al., 2010; Yoshimori, 2004). There are three major forms of autophagy: 1) Macroautphagy, 2) Microautophagy and 3) Chaperone mediated autophagy. During macroautophagy, parts of the

cytoplasm and whole damaged organelles are sequestered into double-membrane autophagosomes (Ghavami et al., 2012a; Rotter and Rothermel, 2012). Macroautophagy could be also involved in sequestration of damaged and misfolded proteins (Gustafsson and Gottlieb, 2009). Following autophagosomes formation, they will fuse with lysosomes to generate single membrane autophagolysosomes, where degradation of their contents occurs (Yoshimori, 2004). Microautophagy refers to direct engulfment of the organelle by lysosomes without formation of autophagosomes (Rotter and Rothermel, 2012). The third form of autophagy, referred to as chaperone-mediated autophagy, is initiated when proteins (mainly cytosolic) carrying a KFERQ amino acid motif are directed by a complex of chaperone proteins to combine with lysosomes, via the latter's LAMP 2A receptors, for engulfment and degradation (Rotter and Rothermel, 2012). The molecular mechanisms that regulate autophagy are not yet fully characterized, however, genetic studies in yeast have identified a set of autophagy-related genes (*ATG*) such as *ATG*5, *ATG*12, *ATG*16, *ATG*8, *ATG*7, which are the major regulators for molecular signaling pathways in autophagy (Ghavami et al., 2012b; Nakatogawa et al., 2009).

Four major steps could be distinguished during the course of autophagy; those are: Initiation, Elongation, Closure and Maturation. Initiation is considered as an autophagospmal membrane isolation step that is driven by an upstream signal (i.e., an ATG14-Beclin 1-hVPS34 complex). The establishment of this complex is required for the formation of phagophores (Abounit et al., 2012; Kang et al., 2011). hVPS34 (phosphatylinositol 3-kinase), one of the key players in the Initiation step, is a member of the class III PI3-kinases. hVPS34 is inhibited by protein (Beclin-1) or chemical [3-methyladenine (3-MA)] factors, which results in disruption of the initiation complex (Wu et al., 2010).

Elongation involves the formation of a double-membrane structure comprised of an ATG512-16 complex and LC3. LC3 is a mammalian homologue of yeast ATG8 (Ghavami et

al., 2011). LC3 is converted to LC3-I via proteolytic cleavage of its C-terminus by ATG4. Then, two E3- and E2-like enzymes, ATG7 and ATG3, respectively, drive the covalent conjugation of phosphatidylethanolamine (PE) to LC3-I, thus forming LC3-II. Similarly, ATG5 becomes covalently bound to ATG12 in the presence of another E2-like enzyme, ATG10. Finally, ATG7 co-ordinates the formation of the ATG5-12-16 complex (Hanada et al., 2007).

Closure consists of final adjustments to LC3-II and ATG5-12-16 in forming the double-membrane autophagosome. LC3-II in the autophagosome membrane interacts with p62, which is a cargo for ubiquitinated misfolded proteins and organelles. LC3-II is extensively used to measure autophagy flux, the rate of formation of autophagosomes, and is considered the "gold standard" for autophagometer scale (Rubinsztein et al., 2009). Maturation is the final step in the autophagic process, and mainly describes the translocation and fusion of autophagosomes with lysosomes to form autolysosomes (Ravikumar et al., 2010). It is here in the autolysosomes that sequestered, damaged proteins and organelles are finally degraded, and their components recycled or disposed of (Abounit et al., 2012).

4.2. Autophagy and Stem Cells

High basal level of autophagy have been reported in human msenchymal stem cells (hMSCs), whereas after differentiation autophagy is largely dowregulated and even undetectable in some cases (Oliver et al., 2012). Hypoxic conditions facilitate MSCs self-renewal (Lee et al., 2013), whereas autophagy inhibition has been shown to facilitate hypoxia induced apoptosis in MSCs (Zhang et al., 2012b). Similarly, high basal autophagy level has been shown in hematopoietic stem (HSCs), dermal and epidermal stem cells (Salemi et al., 2012) with a trend to decrease to a basal level activity after differentiation (Salemi et al., 2012). Different groups have demonstrated that *ATG7* knock down in murine HSCs resulted in decelerated

HSCs self-renewal and negatively affected myeloproliferation (Liu et al., 2010; Mortensen et al., 2011). In contrast, upregulation of autophagy-genes has also been shown during neuronal differentiation in mouse embryonic olfactory bulb (Vazquez et al., 2012). Vazquez and collogues also demonstrated that chemical inhibition of autophagy pathway will block the nervous system development in this animal (Vazquez et al., 2012).

Autophagy also plays a central role in controlling somatic reprogramming. Chen and colleagues (Chen et al., 2011) have shown that autophagy is a positive regulator of pluripotency and that rapamycin facilitates mouse embryonic fibroblast reprogramming to pluripotent stem cells. In this context, autophagy may negatively regulates cellular apoptosis and senescence and therefore positively affecting the reprogramming progress (Menendez et al., 2011). On the other hand, in some experimental systems, mitochondrial targeted autophagy (mitophagy) (Jangamreddy and Los, 2012) facilitates reprogramming via decreasing reactive oxygen species production (Fimia et al., 2007; Vazquez et al., 2012).

4.3. Possible Role of Autophagy in iPS cell Generation

As we have previously indicated, autophagy is a quality control mechanism, which guarantees normal cell function at different stages of cellular life (Jia et al., 2011). Transcription factors Oct3/4, Sox2, c-Myc, and Klf4 are employed to reprogram differentiated cells towards an embryonic-like state (Marchetto et al., 2010), called iPS. Interestingly, iPS cells not only express stem cell markers, but also stem cell characteristics including anaerobic metabolism, low reactive oxygen species generation and decrease in mitochondrial mass and number (Armstrong et al., 2010; Prigione et al., 2010; Suhr et al., 2010). It has been suggested that the decrease in mitochondrial numbers contributes to lower generation of superoxide ions in iPS cells as compared to their progenitors (Vessoni et al., 2012).

Autophagy plays an important role during differentiation of hematopoietic progenitor cells to erythrocytes and adipocytes by selective mitochondrial autophagy (Lafontan, 2008; Singh and Cuervo, 2011). Thus it could be hypothesized that autophagy might also participate in reprogramming of differentiated cells to pluripotent form during iPS generation. The observed lower mitochondria numbers in iPS cells may be achieved by selectively targeting mitochondria (mitophagy). Additionally, autophagy may actively facilitate generation of cells with pluripotent stem cell properties via lysosomal degradation of proteins typical for more differentiated cellular stage. Lastly, autophagy may also play a role during phenotype-conversion process by improving protein synthesis via amino acids recycling. Therefore, autophagy could be considered as a potential mechanism, participating not only during cell differentiation, but also throughout reprogramming, or even transdifferentiation processes.

5. Reprogramming Factors and Carcinogenesis

Cancer cells and stem cells share certain characteristics, like the ability of self-renewal (Reya et al., 2001), and already discussed above expression of certain stemness factors, including Yamanaka factors. Many pathways that are altered in cancer cells regulate functions of embryonic and adult stem cells. Reprogramming factors (Yamanaka factors) are at the same time proto-oncogenes and may directly affect the proliferation. On the other hand, Yamanaka factors expression is associated with a high level of genomic instability and also indirectly affect proliferation. In Table II we list the key genes important for the induction of iPS cells, while also show their function in oncogenic transformation, tumor progression, dissemination or (poor) prognosis.

Besides being part of the Yamanaka reprogramming cocktail, c-Myc and Klf4 are well-known proto-oncogenes, they also play a role in stem-cell self-renewal. Both above

facts emphasize the parallels between the induction of pluripotency and carcinogenesis. The Klf4 encodes a transcription factor that is associated with both tumor suppression and oncogenesis. As transcriptional repressor of p53, Klf4 acts as a context-dependent proto-oncogene (Rowland et al., 2005). Klf4 and c-Myc lead to DNA replication stress and genomic instability. Comparative genomic hybridization analysis of iPS cells has revealed occasional presence of genomic deletions and amplifications, a signature suggestive for oncogene-induced DNA replication stress. The genomic aberrations were to a significant degree dependent on c-Myc expression and their presence could explain why p53 inactivation facilitates reprogramming (Marion et al., 2009). The expression of some oncogenes such as c-Myc in epithelial tumors is sufficient to reactivate an ES cells-like transcriptional signature

Other reprogramming factors, such as Sox2 and Lin28 have been documented as oncogenes in small cell lung and oesophageal squamous-cell carcinomas and germ-cell tumors (Bass et al., 2009; West et al., 2009). Aggressive, poorly differentiated tumors also express high levels of the ES cells-associated factors (Ben-Porath et al., 2008). Lengerke and colleagues have shown that Sox2 is expressed in a variety of early stage postmenopausal breast cancers and associated metastatic lymph nodes. They conclude that Sox2 plays an early role in breast carcinogenesis and high expression may promote metastases (Lengerke et al., 2011). Others have demonstrated that Sox2 alone or Sox2/Oct4A expression in hepatocellular cancer could serve as a prognostic marker, predicting the outcome of the treatment of hepatocellular cancer patients even at an early stage of the diseases. Thus, Sox2 and Oct4A are predictors of poor prognosis for patients undergoing resection of hepatocellular cancer (Huang et al., 2011).

6. Stemness Factors as Transcription Factors - Effects in Transformation and Reprogramming

Functionally, Yamanaka factors are transcription factors that regulate the expression of a number of genes, including some of the reprogramming factors. Below we discuss the regulatory functions of selected Yamanaka factors.

c-Myc proto-oncogene is a transcription factor that regulates a number of genes involved mainly in proliferation and DNA-replication. It is overexpressed in many tumor types, thus not surprisingly, it is also at least partially responsible for tumor formation in tissues derived from iPS cells (Okita et al., 2007). Nakagawa and colleagues (Nakagawa et al., 2008) and Wernig and colleagues (Wernig et al., 2008) showed independently that the iPS cells generation from mouse embryonic fibroblast using only three Yamanaka's factors, omitting c-Myc, is possible; however the lack of retroviral c-Myc does not exclude endogenous c-Myc recruitment in the iPS cells generation process. Interestingly, in Nakagawa *et al.* study 6/37 chimeras derived from iPS cells generated using four Yamanaka's genes died of tumor within 100 days, while all 26 chimeras derived from three factors cocktail (without c-Myc) survived during the 100 days. Also, the iPS cells generation from human adult fibroblast is possible when retroviral c-Myc is absent, although with the lower reprogramming efficiency (Nakagawa et al., 2008). Further attempts to resolve the importance of c-Myc in iPS cells generation clarified that different properties of c-Myc are responsible for carcinogenic transformation and different for the iPS cells generation. Hence, oncogenic c-Myc could be replaced by genetically-engineered c-Myc or other Myc family member L-Myc, which fulfills both criteria (Nakagawa et al., 2010).

Sox2 is a transcription factor belonging to the SOXB1 group of Sry-related HMG box. Sox2 heteredimerizes with another transcription factor Oct4 and together they are important for activating genes important for stem cells maintenance (Lefebvre et al., 2007). Sox2 and Oct4 have been found at high levels in many types of tumors, for example neuroblastoma (Yang et al., 2012), gliomas (Guo et al., 2012), liver cancer (Huang et al.,

2011) and are of importance for the outcome of cancer therapy (Hu et al., 2012; Stolzenburg et al., 2012). However, Cantz *et al.* showed that HeLa and MCF-7 cancer cell lines are Oct4-negative and in accordance, the Oct4 promoter is hypermethylated in those cells (Cantz et al., 2008). Suo *et al.* demonstrated that Oct4 pseudogenes, which are not important for iPS cell generation, in addition to-, or instead of Oct4 gene are variably expressed in different cancer types (Suo et al., 2005) and therefore the role of Oct4 gene in cancrogenesis is debatable.

Klf-4, depending on its target genes, may have multiple, sometimes opposing, roles in regulating cell proliferation, apoptosis and differentiation (Evans and Liu, 2008). Its dual role extends to the cancer cells where Klf-4 can act as both tumor suppressor and an oncogene depending on the cancer type. The first function has been found for example in gastric cancer (Zhang et al., 2012a) or B-cell malignancies (Kharas et al., 2007). The fluctuating Klf-4 levels can also mirror the differentiation state of malignant cells (Guo and Tang, 2012). One of the functions of Klf-4 is suppression of the Wnt/β-catenin signaling. Hence, disturbed Wnt/β-catenin is associated with the tumourigenesis. Klf4-β-catenin interaction is crucial for regulating telomere length (Hoffmeyer et al., 2012). Klf4 also interacts with p53-p21 pathway (Zhang et al., 2000) by downregulating p53 promoter, as described for breast cancer cells (Rowland et al., 2005). The p53 protein is a well-known tumor suppressor and its mutations are frequently associated with the tumor progression. Hong *et al.* showed that the suppression of p53 and p21 results in increased efficiency of iPS cell generation in mice and in human (Hong et al., 2009).

Lin28 and Nanog, although not included in the original Yamanaka's 'cocktail', are currently used in some protocols for the cell reprogramming. Lin28 and its human homologue Lin28B regulate let-7 miRNA by blocking let-7 precursor from processing (Viswanathan et al., 2008). Both the repression of let-7 (Kumar et al., 2008; Nadiminty et al.), and the expression of Lin28 have been associated with tumorigenicity (Viswanathan et

al., 2009). Among other functions, Nanog drives the expression of Oct4. Several studies showed the expression of Nanog in the context of cancer cells (Jeter et al., 2009; Zbinden et al.). However there are eleven human Nanog pseudogenes (Booth and Holland, 2004), which function might differ from Nanog gene and those might be of importance for tumorigenicity. For example, Nanog pseudogene 8 has been associated with tumorigenecity and cell proliferation (Uchino et al., 2012; Zhang et al., 2006).

7. Differences and Similarities between iPS Cells and Cancer Stem Cells

iPS cells exhibit similar behavior not only to stem cells but they also may have some similarities to CSC, particularly with respect to expression of certain stemness factors, and proliferative properties. CSC, so far found in several human solid and hematological tumors, are able to initiate tumor formation and metastasis (Galli et al., 2004; Ribatti, 2012). They are associated with chemo- and radiotherapy resistance and poor patient prognosis (Saigusa et al., 2009). Some of them express specific cell surface markers like i.e. CD133 (Beier et al., 2008; Tirino et al., 2008; Zhou et al., 2007). Moreover, CSC express increased levels of anti-apoptotic proteins in comparison to mature cell types from the same tissue which could explain CSC's resistance to cytotoxic drugs (Bitarte et al., 2011; Ribatti, 2012). Like iPS cells, CSC may have gene expression profile different from respective, normal progenitor cells (Reya et al., 2001), possess high proliferation rate and thus positivity for Ki-67 (Tirino et al., 2008).

CSC and iPS cells exhibit several striking similarities that we summarize in Table III, furthermore iPS cells form teratomas when injected into tissues (Hanley et al., 2010; Miura et al., 2009). Moreover, differentiated tissues that originate from iPS cells have an increased propensity to form tumors. Formation of tumors seems to depend on the origin of cells. iPS cells derived from different adult tissues may vary in their capacities to differentiate into

certain tissues, and may vary substantially in their teratoma-forming propensity (Miura et al., 2009; Yamanaka, 2009). The increased tendency of iPS cell-derived tissues to form tumors may be directly connected to the use of c-Myc in some protocols for generation of iPS cells. Retroviral introduction of c-Myc may results in tumor formation in even ~50% of chimeric mice generated from iPS cells (Okita et al., 2007). Although reprogramming methods exist that do not use c-Myc (Nakagawa et al., 2008; Wernig et al., 2008), they are less efficient, the reprogramming is protracted, and pluripotency is weakened (Yamanaka, 2009). Furthermore, the other three reprogramming factors may also cause tumors (Yamanaka, 2009), and they are often overexpressed in subset of cancer cells (Huang et al., 2011; Lengerke et al., 2011).

Senescence induction during reprogramming is another crucial aspect contributing to lowered efficiency of iPS production and culture, as well as playing pivotal role in cancer stem cells biology. Factors and processes involved in reprogramming evoke cellular senescence and therefore impair successful reprogramming to pluripotent stem cells (Banito et al., 2009). It has been i.e. shown that human iPS-derived early blood progenitor cells appear to undergo premature senescence (Feng et al., 2010). It was also suggested that in order to control the efficiency of iPS generation, senescence may be activated by tumor suppressors like p53 and p16(INK4a) (Banito and Gil, 2010).

CSC seem to behave in a distinct way. CSC found among MCF-7 breast cancer cells showed significantly reduced propensity to undergo senescence, which could be a result of increased telomerase activity and a low level of p21 protein. These cells also had a reduced level of reactive oxygen species, and more active DNA single-strand break repair pathway (Karimi-Busheri et al., 2010). Sabisz and Składanowski observed in turn, that A549 cells treated with DNA damaging antitumor drugs revealed premature senescence, however a small fraction of them (CSC) escaped from senescence and lead to re-growth of tumor cell population (Sabisz and Skladanowski, 2009).

One of the most intriguing issues concerning iPS cells involves the fact that p53 may prevent pluripotency. It turns out that when p53 is damaged, or deleted from cell genome - cells more easily become iPS cells (Hong et al., 2009; Kawamura et al., 2009), and the generation of iPS cells may increase even up to 20% in cells without p53 (Hong et al., 2009). p53 loss mainly contributes to reprogramming by accelerating cell cycle progression (Hanna et al., 2009). To enhance the reprogramming of cells one may target p53 pathway in a safer way, by carrying-out the process under hypoxic conditions (Bae et al., 2012; Yoshida et al., 2009) or in the presence of ascorbic acid (Esteban et al., 2010). Alternatively, inhibition of p53 is achieved by the increase of expression of SV40 large T antigen or Rem2 GTPase (reviewed in (Stadtfeld and Hochedlinger, 2010)). However, both manipulations carry an increased risk of oncogenic transformation.

Undoubtedly loss of p53 plays also an important role in CSC biology. Several scientific groups reported that CSC derived from p53-null mice have a high tumor-initiating frequency (Zhang et al., 2008) and link the loss of p53 function to increased expression of CD44, which promotes expansion of tumor-initiating cells (Godar et al., 2008). These articles suggest that loss of p53 permits expansion of presumptive CSC especially in mouse mammary tumors and in human breast cell lines. Suppression of cancer stem cell phenotype is an additional activity by which p53 can inhibit tumors.

CSC, as already mentioned, are often resistant to DNA damage inducers, and show greater DNA repair capacity than their non-stem-like counterparts (Bao et al., 2006). For example, CSC contribute to radioresistance of glioma cells through preferential activation of the DNA damage checkpoint response and an increase in DNA repair capacity. Tumor cells expressing CD133 (a marker for brain [cancer] stem cells) survived ionizing radiation and their fraction was enriched in gliomas following ionizing irradiation (Bao et al., 2006). However, human iPS cells exhibit hypersensitivity to DNA damaging agents following

gamma-irradiation, which results in rapid induction of apoptosis (Momcilovic et al., 2010). Expression of pluripotency factors in iPS cells did not appear to be diminished after irradiation (Momcilovic et al., 2010). Moreover, high degree of similarity in DNA damage responses between iPS cells and ES cells were found. After irradiation, similarly to ES cells, iPS cells activated checkpoint signaling, evidenced by phosphorylation of ATM, NBS1, CHEK2, TP53 and localization of ATM to the double strand breaks (DSB) (Momcilovic et al., 2010).

Both CSC and iPS cells have altered cell cycle as compared to normal and stem cells. Experiments carried out by Ghule and coworkers revealed that human iPS cells have a short G1 phase and an abbreviated cell cycle (16–18 h). Furthermore, histone locus bodies are formed and reorganized shortly after mitosis within 1.5–2 h (Ghule et al., 2011). Other results suggest in turn that the expression of reprogramming factors increase the percentage of cells arrested in G1 without significant induction of apoptosis (Banito et al., 2009). It was also reported that exposure of iPS cells to DNA damaging agents result in arrest of cell cycle progression in the G2 phase and the population display high percentage of cells in S phase (Momcilovic et al., 2010).

CSC subpopulations of epithelial, breast and prostate carcinoma are characterized by high proportion of cells in the G2 phase, which may suggest that they spend a consistently longer time in G2 (Harper et al., 2010). Longer G2 phase was also observed by Chappell and Dalton (Chappell and Dalton, 2010) suggesting longer time for DNA repair in CSC.

Growing body of evidence collected mainly in the last three decades show important role of epigenetic changes in carcinogenesis, and cancer progression (Hellebrekers et al., 2007; Rius and Lyko, 2012). Low resolution scanning of genomes of ES-cells IPS cells and cancer cells revealed largely overlapping but not identical pattern of DNA methylation (Doi et al., 2009).

8. Interaction of Biomaterials with Cells

Some earlier biomaterials tested in animals have caused higher incidence of oncogenic transformation within the surrounding tissues (Denishefsky et al., 1967). One possible mechanism responsible for such events is Ras pathway activation *via* the interaction of integrins (types β_1, $\alpha_V\beta_3$ or $\alpha_6\beta_4$) with their ligands and subsequent activation of Shc (Src homology 2 domain-containing). Activated Shc in turn triggers the Ras/MAPK signaling cascade that then alters gene expression (Wary et al., 1996), sometimes leading to oncogenic transformation (please see below). Therefore, we fell that a dedicated chapter covering interactions of biomaterials with cells is needed.

Due to pathologies or traumatic injuries, organs or tissues can lose their function. Thus, there is a large need for engineered replacements, including joints, heart valves, corneas and intraocular lenses made from a number of different biomaterials (Binyamin et al., 2006; Hench, 1980). A biomaterial can be defined as any substance, synthetic or natural in origin, that is used to replace or restore function to a body tissue or organ and maybe continuously or intermittently in contact with body fluids, cells, and tissues, and can be divided into ceramics, polymers, metals, and composites (Binyamin et al., 2006). Initially, research focused on producing biomaterials that did not elicit a biological response from the host, that is, a bioinert material. By the 80s a shift developed, where new biomaterials were designed to interact with the surrounding cells and tissues (Hench and Wilson, 1984). This include biomaterials that mimic the extracellular matrix (ECM) or release soluble factors that influence activity of the surrounding cells (Hench and Thompson, 2010; Place et al., 2009).

Orthopedic replacements should be capable of supporting functional loading and for this reason, metal biomaterials are often used due to their excellent mechanical strength (Zreiqat et al., 2005). A commonly used bioinert metal biomaterial, are close to being

bioinert titanium alloys (Ti-6Al-4V) (Zhao et al., 2012). In order to sustain functional loading, the implant should be anchored to the bone, through the actions of osteogenic cells. It was found that osteoblasts were able to adhere to titanium through production of ECM proteins that was absorbed onto the surface of titanium (Matsuura et al., 2000). The quantities of ECM proteins produced were, however, insufficient while some proteins were not produced at all, leading to inadequate bone anchorage. To improve the integration of implant into the bone, it has been attempted to alter the surface of metal biomaterials to combine the excellent mechanical features of metal biomaterials with a bioactive surface. Examples of bioactive surfaces includes hydroxy-apatite, that is the main mineral component of normal bone and Bioglass (Hench and Thompson, 2010; Zreiqat et al., 2005). Bioglass is a type of bioactive glass consisting of Na_2O, CaO, P_2O_5 and SiO_2. When Bioglass is implanted into bone tissue an ion exchange between the Bioglass surface and the surrounding fluids initiates. This ion exchange leads to absorbance of Ca, PO_4 and CO_3 that later crystallize to form hydroxyl-carbonate apatite. Surrounding osteoblasts will then integrate the newly formed Bioglass surface with the surrounding bone. Bioglass has been bound to the surface of titanium implants, where the Bioglass allows strong integration of the titanium-Bioglass implant (Hench and Thompson, 2010). The effects of different orthopedic or dental biomaterials on osteoblast function are presented in Table IV. In summary both hydroxy-apatite coating and Bioglass positively affect the bone integration of implants (Hench and Thompson, 2010; Zreiqat et al., 2005).

Bioactive surfaces have been investigated as a tool in tissue engineering, with much of the research being focused on mimicking the ECM. Biomaterials based on components of the ECM may direct differentiation of pluripotent stem cells towards different lineages (Table V) (Dickinson et al., 2011). Besides serving as structural support, the ECM provides biochemical and mechanical signals to the cells, mainly mediated by integrins, which helps

control cell fate (Eshghi and Schaffer, 2008). Integrins are transmembrane proteins that serve to link the intracellular compartment with the ECM. Binding the ECM components vitronectin and fibronectin with integrins of the types β_1, $\alpha_V\beta_3$ or $\alpha_6\beta_4$ activates the cytosolic protein Shc resulting in activation of the Ras/MAPK signaling cascade that in turn alters gene expression (Wary et al., 1996). Integrins bind to small peptides in the ECM adhesive components, termed RGD (arginine-glycine-aspartic acid) (Ruoslahti and Pierschbacher, 1987). Using phosphonic acid groups as linker molecules, titanium may be covalently covered with RGD peptides. These peptides bind to $\alpha_V\beta_3$ integrins and engage the subsequent Ras/MAP signaling cascade, inducing adhesion of osteoblasts to the implant. These events also subsequently induce the proliferation of fibroblasts and osteogenic differentiation resulting in a strong incorporation of the implant into the surrounding bone (Auernheimer et al., 2005).

Despite the obvious advantages of using biomaterials, negative reactions have been noted. While traditional biomaterials strived to reach bioinertness, most of them do produce some biological foreign body response. Zhao *et al* found that osteoblasts reacted to supposedly bioinert biomaterials like titanium alloys by an increased production of inflammatory proteins (Table V) (Zhao et al., 2012). This manifested as formation of a fibrous cap that separated the implant from the surrounding tissues. Since the biomaterial and the fibrous cap do not adhere to each other, this severely weakens the ability of the implant to withstand mechanical loading (Hench, 1980).

During the 1950s and 1960s studies indicated that mice and rats that were implanted with solid materials including plastic, metals and glass, had a high risk of developing sarcoma (Denishefsky et al., 1967). The originator cells of the sarcoma was eventually isolated and identified as pericytes, which are pluripotent stem cells serving as progenitors of small blood vessels. Development of a fibrous capsule surrounding the implant required

neovascularisation that is why the pericytes proliferated. Hyperplasia of the pericytes increases the risk of developing genetic errors, which could result in formation of precarcinogenous cells and eventually malignancies. Despite the findings in rodents, no human cases of foreign body related cancers where known at that time (Brand, 1994). Newer studies focused on metals like cobalt, iron, nickel and chromium that are released to the body by metal biomaterials like hip replacements, and can be found in liver, lymph nodes and spleen (Case et al., 1994). Chromium ions pass the cell membranes through membrane anion transporters. In the cell, chromium is reduced and can form covalent interactions with the DNA, thereby causing double strand DNA breaks (Singh et al., 1998). Thus chronic chromium exposure has been linked with an increased risk of malignancies (Parry et al., 2010; Tsaousi et al., 2010).

9. Closing Remarks

The rapidly developing field of regenerative medicine will certainly revolutionize clinical handling of numerous diseases in not so distant future. In medicine and biomaterials science, there has been a paradigm shift from replacement to regeneration of tissues & organs, and consequently a shift from synthetic to natural-based biomaterials to enhance the interactions between the body and biomaterials. Therefore, it is critical to gain sufficient understanding about the nature and consequences of interactions between biomaterials introduced to the body and the induced biologic responses. As outlined above, the focus of dedicated research has been mostly on an inflammatory reaction and possible carcinogenic effects of biomaterials. There is an urgent need to look into those interactions at a mechanistic level. For example, (*i*) how cell death and cell survival processes are affected by those interactions (Jain et al., 2013; Jangamreddy and Los, 2012; Maddika et al., 2007), (*ii*) if- and how certain genetic markers may influence carcinogenic process in the vicinity of implanted biomaterials

(Denishefsky et al., 1967; Wiechec, 2011; Wiechec et al., 2013), and (*iii*) what are the long-term effects of coexistence of biomaterial and cells. It would be unsafe to use iPS cells therapeutically until we fully understand these complex relations between pluripotency reprogramming and carcinogenesis. New generation of anticancer drugs that preferentially target CSC (Jangamreddy et al., 2013), may help decreasing the risk of transplant induced malignancies.

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Table I: Advantages/disadvantages of different reprogramming techniques/vectors.

Vector	Advantages	Disadvantages
Lentivirus	Sustained expression of reprogramming factors (Hotta and Ellis, 2008). Can be used to transduce both proliferating and non-dividing cells (Patel and Yang, 2010).	Insertion mutagenesis which may lead to cancer (Yu et al., 2009).
Adenovirus	Transient expression causes removal of viral vector following reprogramming (Okita et al., 2008). No genomic integration thus preventing insertional mutagenesis (Okita et al., 2008).	Low reprogramming efficiency (Okita et al., 2008). Requires repeated transduction in order to successfully reprogram fibroblasts (Okita et al., 2008).
Sendai virus	Efficient introduction of genomic material into host cells (Fusaki et al., 2009). Can affect several types of cells (Fusaki et al., 2009). No genomic integration thus preventing insertional mutagenesis (Fusaki et al., 2009).	Transfected cells tend to contain viral remains following transduction, that requires additional techniques for removal (Okita and Yamanaka, 2011).
Plamids	Long-term transient gene expression allows reprogramming with only a single transfection (Yu et al., 2009). Episomal gene expression prevents insertional mutagenesis (Yu et al., 2009). Plasmids are lost following repeated culturing (Yu et al., 2009). Can be used in a wide variety of cells (Yu et al., 2009).	Low transfection efficiency (Fusaki et al., 2009). Succesfull reprogramming requires several plasmids (Jia et al., 2010).
Minicircle	Higher transfection efficiency than plasmids (Jia et al., 2010). Unlike standard plasmids, minicircles does not contain bacterial DNA (Jia et al., 2010). Can reprogram somatic cells using only one vector (Jia et al., 2010).	Low transfection efficiency compared t viral techniques (Jia et al., 2010; Okita et al., 2008).

	Longer expression that plasmids (Jia et al., 2010). Minicircle vectors are lost following repeated culturing (Jia et al., 2010). Can be used in a wide variety of cells (Jia et al., 2010).	
Piggybac transposon	Sustained expression allow for a higher reprogramming efficiency (Woltjen et al., 2009). Reprogramming vectors can theoretically be removed to produce vector free iPS cells (Woltjen et al., 2009).	Cells might contain remains of vector following reprogramming (Jia et al., 2010). Low reprogramming efficiency compared to viral techniques (Jia et al., 2010; Okita et al., 2008).
mRNA	No introduction of ectopic DNA (Plews et al., 2010). The small size of mRNA molecules results in a high introduction into the cells with a low cytotoxicity (Plews et al., 2010). Better control of the reprogramming (Plews et al., 2010). Good reprogramming efficiency (Okita and Yamanaka, 2011).	mRNA is degraded within two or three days. As such, repeated transfection is required for successful reprogramming (Plews et al., 2010).
Proteins	No introduction of ectopic nucleic acids (Kim et al., 2009a).	Proteins produced by bacteria might be misfolded or lack essential modifications (Plews et al., 2010). Proteins provided as cell lysatecontains several poorly defined substances beside the reprogrmming factors (Plews et al., 2010) Low reprogramming efficiency (Kim et al., 2009a).

Table II: Examples of the cancer types in which transcription factors important for cell reprogramming are expressed.

Reprogramming factors	**Cancer type *& observed association***	**References**
Sox2	Nasopharyngeal cancer *Clinical prognosis*	(Wang et al.)
	Melanoma *Cell invasion*	(Girouard et al.)
	Colon cancer *Cell invasion and metastases*	(Neumann et al.)
	Osteosarcomas *Survival and self-renewal*	(Basu-Roy et al.)
	Gastric cancer	(Matsuoka et al., 2012)
	Lung cancer *Transformation*	(Chen et al.)
	Prostate cancer	(Jia et al.)
	Breast cancer	(Stolzenburg et al.)
Oct4	Gastric cancer *Negative prognostic factor*	(Matsuoka et al., 2012)
	Oral squamous cell carcinoma	(Chiou et al., 2008; Tsai et al.)
Klf4	Breast cancer *Good prognosis*	(Nagata et al.)
	Leukemias *Different Klf4 mRNA levels mirror differentiation state, not a prognostic factor*	(Guo and Tang, 2012)
	Squamous cell carcinoma *Poor prognosis*	(Chen et al., 2008)
	Breast cancer	(Rowland et al., 2005)

Nanog	Hepatocellular carcinoma	(Shan et al.)
	Breast cancer – *Poor prognosis*	(Nagata et al.)
	Oral squamous cell carcinoma (OSCC) (Nanog/Oct-4/CD133 coexpression) *Poor prognosis*	(Chiou et al., 2008)
Lin28	Breast cancer *Poor prognosis*	(Feng et al.)
	Transformation	(Viswanathan et al., 2009)
	Lung cancer *Transformation*	(Viswanathan et al., 2009)
	Colon cancer *Transformation*	(Viswanathan et al., 2009)
	Cervical cancer *Transformation*	(Viswanathan et al., 2009)
	Germ cell tumours *Transformation*	(West et al., 2009)
	Extragonadal germ cells tumours *Transformation(diagnostic marker)*	(Cao et al.)
	Ovarian cancer (co-expressed with Oct4) *cell growth and survival*	(Peng et al.)

Table III: Differences and similarities between iPS and cancer stem cells.

Feature	iPS cells	Cancer stem cells
Tumor formation	Increased propensity to form teratomas (Hanley et al., 2010; Miura et al., 2009)	Forms tumors by definition (Galli et al., 2004; Ribatti, 2012)
p53	p53 loss increases pluripotency (Hanna et al., 2009; Hong et al., 2009; Kawamura et al., 2009)	p53 loss increases tumor initiation frequency (Godar et al., 2008; Zhang et al., 2008)
Senescence	Premature senescence (Feng et al., 2010)	Reduced senescence (Karimi-Busheri et al., 2010; Sabisz and Skladanowski, 2009)
Cell cycle	Short G1 phase, abbreviated cell cycle (Ghule et al., 2011), arrest in G2 phase after DNA damage (Momcilovic et al., 2010)	Long G2 phase, high proportion of cells in the G2 (Chappell and Dalton, 2010; Harper et al., 2010; Tirino et al., 2008)
Treatment resistance	Hypersensitivity to DNA damaging agents, induction of apoptosis (Momcilovic et al., 2010)	Resistance to DNA damaging agents, efficient DNA repair (Bao et al., 2006)
Gene expression	Small differences between iPS cells and ES cells gene expression (Yu et al., 2009)	Gene expression different in CSC and tumor cells (Reya et al., 2001)

Table IV: Genes up-regulated in osteoblasts in response to the surface of different biomaterials and the effects.

Biomaterial	Protein/gene	Effect	Reference
Titanium alloy	Vinculin	Formation of focal adhesions leading to stronger cell adhesion to biomaterial	(Räisänen et al., 2000)
	NDRG1	Marker of stress response	(Zhao et al., 2012)
	TGFβI	Stimulate cell migration while inhibiting cell adhesion	
	Ferritin	Intracellular storage of iron. Up-regulate pro-inflammatory mediators	
Hydroxyappatit coating	β_1 integrin	Activates Shc in response to integrin-ECM binding	(Zreiqat et al., 2005)
	Shc	Stimulates RAS/MAPK signaling	
Gold	Vinculin	Formation of focal adhesions leading to stronger cell adhesion	(Räisänen et al., 2000)
	$\alpha_6\beta_4$ integrin	Activates Shc in response to integrin-ECM binding	
Bioglass	RCL	Initiate cell cycle	(Xynos et al., 2000)
	Cyclin D	Causes progression from G1 phase to S phase of the mitosis	
	Cyclin K		
	CDKN1A		
	Insulin like growth factor II	Osteoblast homeostasis	
	VEGF	Enhances osteogenic differentiation of osteoblasts	
	β_1 integrin	Activates Shc in response to ECM components	
	MAPK	Components of the MAPK signaling pathways, thus indicating activity of the MAPK signaling cascade	
	MAP2K		
	MAPK3		
	CD44	Marker of osteocytic differentiation	

Table V: Differentiation of pluripotent stem cells in response to biomaterials coated with different ECM components.

ECM component	Differentiation of pluripotent stem cells	Reference
Collagen I	Cardiomyogenic lineage	(Sato et al., 2006)
Collagen IV	Haematopoetic cells Smooth muscle cells	(Schenke-Layland et al., 2008)
Laminin	Neural cells Pancreatic islet cells	(Dickinson et al., 2011)
Fibronectin	Mesodermal and ectodermal lineages	(Dickinson et al., 2011)

11. Literature

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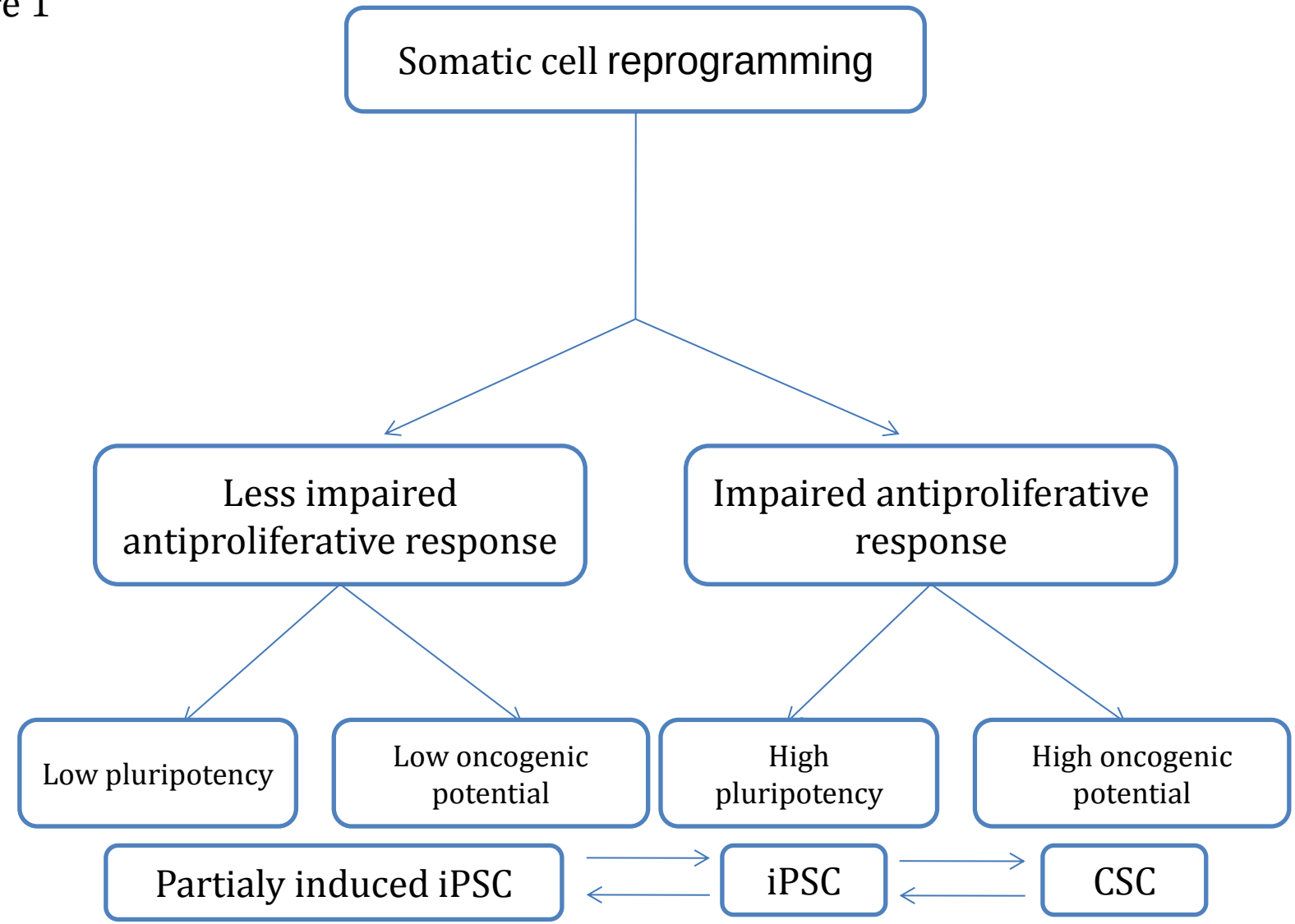
Figure legend

Figure 1: The relationship between antiproliferative response, pluripotency and oncogenesis. Antiproliferative response is activated during reprogramming. At the same time expression of reprogramming factors impairs antiproliferative response by causing mutations on the main tumor supressor genes. This impairment makes reprogramming more susceptible but increase the possibility for oncogenic transformation. Cell commitment depends on this complex interplay.

Figure 1


Somatic cell reprogramming
Less impaired antiproliferative response
Impaired antiproliferative response
Low pluripotency
Low oncogenic potential
High pluripotency
High oncogenic potential
Partialy induced iPSC
iPSC
CSC