Chapter 6
In Vivo Reprogramming Towards Pluripotency for Tissue Repair and Regeneration

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6.1 Introduction

A series of proof-of-principle studies demonstrated at the beginning of this decade that cells within adult, fully differentiated tissues can be directly reprogrammed in situ to acquire several hallmarks of pluripotency, including the capacity to proliferate. This process is induced by overexpression of defined transcription factors—the combination of *Oct3/4*, *Sox2, Klf4* and *cMyc*, also known as OSKM—that can reset the primitive plasticity of undifferentiated cells in spite of the presence of pro-differentiation signals that naturally govern the adult tissue microenvironment [1–4]. Since then, the induction of pluripotency in vivo via OSKM overexpression has been used to further unravel the mechanisms behind reprogramming, as well as to investigate its connections with other cellular processes, including the onset of tumorigenesis and cellular senescence (see Chap. 5 of this book). However, beyond the invaluable role of in vivo reprogramming models as research tools to answer the questions above, the therapeutic applications that could be developed from this strategy, in particular to induce or enhance tissue regeneration, have also been envisioned. In this Chapter, we discuss the rationale behind the use of in vivo reprogramming towards pluripotency to assist tissue repair. We also analyse opportunities and challenges on the road towards clinical translation and review the studies, although scarce, that have already confirmed the potential of in vivo reprogramming to pluripotency to enhance the regenerative capacity of injured and degenerated tissues.

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6.1.1 Hypothesis: Generation of an In Situ Source of De Novo Cells to Repair Injured or Aged Tissues

A plethora of conditions and insults to the organism trigger the loss of specific cell populations, some of which cannot be efficiently replenished by the adult mammalian organism. Such is for example the case after myocardial infarction, whereby the ischemic accident leads to death of a large number of cardiomyocytes. Current treatments can only help the heart adapt to the new situation by decreasing its workload and thus minimise the risk of future ischemic events, but fail to induce the generation of new cardiomyocytes that restore the intact function of the organ [5]. Similarly, lack of blood supply to certain parts of the brain during ischemic or haemorrhagic stroke results in loss of neuronal cells that cannot be replaced [6].

Direct reprogramming of surviving cells within and injured or degenerated tissue via in vivo OSKM overexpression has been proposed as a novel strategy to induce or enhance its repair and regeneration [7]. Thanks to their capacity to proliferate but also to re-differentiate back into mature phenotypes, in vivo reprogrammed cells could be used as an in situ source of de novo cells to replenish those lost upon injury or degeneration. Such hypothesis is illustrated in Fig. 6.1. While OSKM factors have sufficiently proven their capability to induce de-differentiation in a variety of cell types in vitro and in vivo, it is expected that pro-differentiation cues present in the tissue microenvironment are able to drive re-differentiation of reprogrammed intermediates into fully functional mature cell types [7]. Experimental evidence that supports this hypothesis has been provided in studies that followed the re-differentiation of reprogrammed intermediates, as well as their re-integration in the host tissue and accomplishment of their physiological function [8].

6.1.2 Lessons Learnt from Nature: De-Differentiation for Regeneration

Induction of cell de-differentiation and proliferation is in fact not a new tool to attain tissue regeneration, at least in the context of lower species that are tremendously efficient at regenerating injured tissues, lost appendages and significant portions of vital organs. In zebrafish, heart regeneration is mediated by cardiomyocyte de-differentiation and proliferation [9]. In the newt, proliferating cells that originate from de-differentiated myofibers contribute significantly to form the blastema that precedes limb regeneration [10].

It is not clear whether such regenerative mechanisms have been completely abolished in the mammalian organism as result of evolution, or simply silenced and dormant [11]. A window of efficient heart regeneration via cardiomyocyte proliferation is indeed reported in the neonatal mouse heart, but such capacity vanishes after the first week of life [12]. In the adult, studies have pointed at a very limited degree of cardiomyocyte turnover [13], by far insufficient to provide efficient regeneration,

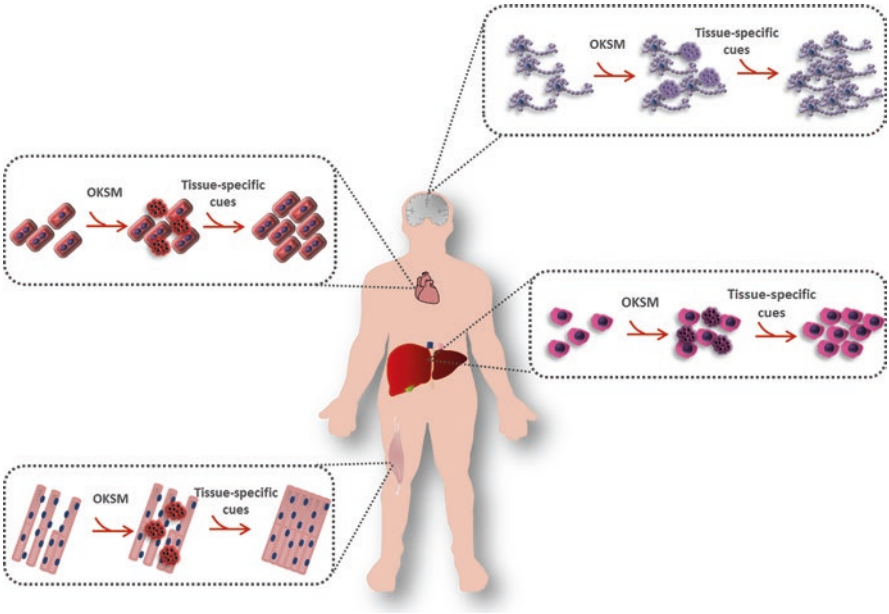

Fig. 6.1 In vivo reprogramming to pluripotency for tissue regeneration. In vivo overexpression of OSKM factors drives reprogramming of a wide variety of starting cell types to a pluripotent-like and proliferative state. It is hypothesised that tissue-specific cues present in the host's microenvironment will be able to orchestrate re-differentiation of the pluripotent intermediates towards appropriate cell phenotypes. Generation of in vivo reprogrammed cells via overexpression of a "universal" cocktail of transcription factors (OSKM) could therefore contribute to enhance regeneration of a variety of injured tissues without the need for ex vivo cell manipulation

and, in addition, the mechanisms by which new cardiomyocytes are generated are yet to be elucidated [14]. Leaving aside rare examples in which spontaneous de-differentiation followed by active division of specific cell populations has been described in vivo [15], the mammalian organism has a lot to learn from lower species.

Overall, regenerative mechanisms in lower species and those still present at the earliest stages of mammalian development have inspired extensive research aiming to recapitulate them in the adult. Many have tried to force or silence the expression of transcription factors and non-coding RNAs known to induce de-differentiation and replenishment of defined cell types in regenerating organisms. Examples of this include exogenous expression of *msx1*, which drives de-differentiation of muscle fibers in urodele amphibians [16] and downregulation of miR99/100 and Let-7a/c, known to induce de-differentiation and proliferation of cardiac myocytes in zebrafish [17]. The role of the Hippo pathway in cardiac regeneration, among other mechanisms, is also under intensive study in the attempts to translate the regenerative capacity of the neonate to the adult mammalian organism [18]. In vivo reprogramming via OSKM overexpression has more recently been proposed as a new alternative in the regenerative medicine portfolio [7, 19].

6.2 In Vivo OSKM Overexpression to Enhance Regeneration After Injury

In spite of the very premature stage at which OSKM-mediated in vivo reprogramming stands today, two independent studies have already provided evidence of enhanced regeneration following administration of the reprogramming cocktail and subsequent generation of pluripotent-like intermediates within injured tissues. Importantly, such evidence has been generated in the context of two distinct injury models that involve different organs, namely traumatic brain injury [20] and skeletal muscle injury [21].

Direct intracranial injection of retroviral vectors encoding OSKM after a controlled cortical impact in mice allowed targeting reactive glia, thanks to the transduction capability of the vectors which is restricted to dividing cells. Indeed, the effect of brain trauma on activating proliferation and migration of glial cells makes them an excellent starting cell source for the generation of in vivo iPS cells via in vivo reprogramming, given their abundance in the injured site. Transduced cells showed hallmarks of pluripotency, including expression of pluripotency marker NANOG and stem cell marker SSEA4, and proliferated actively generating cell clusters that filled the cavity left by the impact. Some reprogrammed cells were found to re-differentiate into neurons and glia, which reassures the potential of this strategy to regenerate the injured brain. However, uncontrolled expansion of reprogrammed cell clusters, caused by the use of integrating gene delivery vectors that sustain long-term expression of reprogramming factors, triggered the generation of teratomas in the brain and therefore compromises any therapeutic application of this strategy as currently designed [20].

In mouse skeletal muscle, forced expression of the same factors encoded in a pDNA cassette via direct intramuscular injection also triggered the appearance of proliferating cell clusters that expressed several pluripotency markers (NANOG, AP, SSEA1) and a marker specific to muscle progenitors (PAX3). However, OSKM expression was not sustained over time, most likely due to the use of an episomal vector that was progressively lost with cell division. As a result, clusters of reprogrammed, pluripotent-like cells were only observed up to 4 days after administration of reprogramming factors and no teratomas were found for the duration of the study (120 days). Morphometric analysis suggested that, on the contrary, in vivo reprogrammed cells could have re-differentiated and fused to existing myofibers, enlarging their calibre. In a clinically-relevant model of severe muscle injury—that involved complete transection of the medial head of the mouse gastrocnemius—OKSM administration accelerated regeneration, as evidenced by the increased numbers of centro-nucleated, small calibre myofibers soon after pDNA administration. Moreover, in vivo reprogramming also showed to prevent excessive collagen deposition, one of the most challenging complications involved in severe muscular injuries that impedes complete recovery of contractile properties [21].

Both studies have opened multiple questions to be answered, not only concerning the safety of the approach but also with regards to the efficiency of reprogramming

achieved and whether this could be sufficient to translate into functional regeneration—not limited to the histological level as in the studies above. Nevertheless, both have offered sound proof of the potential of in vivo reprogramming to enhance regeneration.

6.3 OSKM Overexpression to Rejuvenate Aged Tissues

Induction of tissue regeneration after injury may not be the only therapeutic application provided by the expression of OSKM in vivo. In fact, short but cyclic expression of these factors induces a certain degree of epigenetic remodelling, considered as "partial reprogramming", that does not attain pluripotency but erases several hallmarks of ageing. This event, which may be seen as a strategy for cell "rejuvenation" at the molecular level, could be of interest for the treatment of age-related pathologies. In a mouse model of progeria — a disease in which the onset of ageing is aberrantly premature — cyclic OSKM expression extended the otherwise short life expectancy of the mice and improved the overall condition of various organs and tissues. Even in physiologically aged mice (without the disease), the resilience of aged tissues to injury, impaired compared to that of younger counterparts, increased when OSKM was administered before the insult, following the same cyclic induction protocol. Such improved performance after injury is thought to be achieved through OSKM-driven proliferation of specific cell compartments in charge of tissue turnover and homeostasis, whose numbers normally plummet with age, and was confirmed to occur in two distinct organs. Prior-to-injury expansion of beta cells helped restore glucose tolerance and pancreatic function in a streptozocin model of metabolic disease. In skeletal muscle, satellite cell proliferation prior to intramuscular administration of cardiotoxin, a venom commonly utilised to mimic muscle injury, significantly enhanced tissue regeneration [19].

The observations made in this study confirm that different OSKM induction protocols trigger distinct downstream effects in the tissues, particularly in what concerns the fate of reprogrammed cells. This will be further discussed in Sect. 6.5.1 of this chapter. In addition, it has also become apparent that complete reprogramming to naïve pluripotency may not be a requirement to enhance tissue repair and regeneration in all scenarios [19].

6.4 Opportunities Brought by In Vivo Reprogramming Towards Pluripotency to the Regenerative Medicine Toolbox

In vivo reprogramming via OSKM expression is only one of the numerous strategies currently under preclinical evaluation to achieve efficient cell and tissue regeneration in the adult mammalian organism. However, this particular approach has

attracted increased attention thanks to the promise that it could offer a versatile tool to induce regeneration in virtually any tissue type or organ while avoiding the complications linked to ex vivo cell therapy.

6.4.1 OSKM: A Universal Recipe to Induce Reprogramming Towards Pluripotency

Induction of a more plastic, de-differentiated or pluripotent-like status is not the only option in the in vivo reprogramming toolbox that may be used to enhance tissue regeneration. The number of pre-clinical studies that rely on the concept of in vivo transdifferentiation — direct reprogramming between two distinct mature cell types, for example, fibroblasts to cardiomyocytes — is in fact more abundant in the current scientific literature. One of the reasons behind the popularity of this approach is the bypassing of the pluripotent state, which is understood to minimise the risk of tumorigenesis. In addition, use of cell type-specific transcription factors may provide better control over the resulting phenotype, whereas in vivo reprogramming via OSKM overexpression necessarily relies on molecular cues present in the host's tissue to drive re-differentiation towards appropriate cell types [22].

However, in spite of the undeniable advantages of the transdifferentiation approach, versatility of the OSKM cocktail to induce reprogramming in a wide variety of starting cell and tissue types may save significant research efforts and time and should not be underestimated [7]. Indeed, induction of a particular transdifferentiation event requires identification of specific transcription factors that trigger the precise switch between cell types. The many different combinations of transcription factors utilized in in vivo transdifferentiation studies have been a topic of extensive review [22] and are compiled in Table 6.1. On a complete opposite scenario, OSKM has proven able to induce de-differentiation of a large number of different cell types, from different developmental origins and in different maturation stages; even if significant differences in reprogramming efficiencies have been reported. This has not only been illustrated in the culture dish, where iPS cells have been generated from skin fibroblasts [23], peripheral blood cells [24], liver and stomach cells [25] and pancreatic beta cells [26], among others; but also in vivo. Through ubiquitous OSKM expression in reprogrammable mice, it has been confirmed that in vivo iPS cells can be generated from diverse starting cell types, from haematopoietic and non-haematopoietic origin [4], although different tissues may require different OSKM induction levels to undergo efficient reprogramming [27]. Thanks to this versatility, in vivo reprogramming via OSKM overexpression has already been used to enhance regeneration within very distinct tissue types, namely pancreas [19], skeletal muscle [19, 21] and brain [20].

Table 6.1 Transcription factors (TF) that mediate in vivo transdifferentiation

Starting cell type	Resulting cell type	TF cocktail	Reference
Cardiac fibroblast	Skeletal myofiber	*MyoD*	[28]
	Cardiac myocyte	*Gata4, Mef2c, Tbx5*	[29, 30]
		Gata4, Hand2, Mef2c, Tbx5	[31]
		miRNA1, 133, 208, 499	[32, 33]
Ventricular cardiomyocyte	Pacemaker cell	*Tbx18*	[34, 35]
Exocrine pancreatic cell	Insulin-secreting β cell	*Pdx1, Ngn3, MafA*	[36]
Liver cell	Insulin-secreting cell	*Pdx1*	[37–39]
		neuroD, β-cellulin	[40]
		Pdx1/VP16, NeuroD, Ngn3	[41]
		Ngn3	[42]
		Pdx1, Ngn3, MafA	[1, 43]
Astrocyte	Neuroblast	*Sox2*	[44–46]
	Neuron	*Ascl1, Brn2a, Myt1l*	[47]
		NeuroD1, Ascl1, Lmx1A, miR218	[48]
Fibroblast		*Ascl1, Brn2a, Myt1l*	[47]
Glial cell		*NeuroD1*	[49]
Oligodendrocyte		miRNA 4	[50]
Post-mitotic callosal neuron	Corticofugal neuron	*Fezf2*	[51]
L4 post-mitotic neuron	L5 neuron	*Fezf2*	[52]

In vivo transdifferentiation studies published to date are compiled in this table (updated May 2017), including the specific transcription factors required to trigger each particular switch in cell fate

6.4.2 *Direct In Vivo Reprogramming to Avoid the Challenges of Ex Vivo Cell Therapy*

In the event of cell loss upon injury or degeneration that cannot be addressed by physiological tissue homeostasis, cells grown and/or manipulated in the laboratory can be transplanted to repopulate the injured site. Strategies of this sort have been explored for a number of years, long before in vivo reprogramming and transdifferentiation were considered in the regenerative medicine portfolio, and rely on several sources of replacement cells including embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and iPS cells, within a very extensive list [53, 54]. However, all such cell therapies involve a series of common hurdles related to ex vivo cell manipulation, which complicate their establishment in routine clinical practice, and that could be bypassed by directly inducing cell reprogramming in vivo.

Firstly, donor cell isolation encompasses complications of different magnitudes based on specific cell sources. Use of ESCs involves ethical and regulatory constraints

linked to the destruction of embryonic material [55, 56]. Invasive biopsy techniques are required to access certain population of progenitor and adult stem cells [57]. The least problematic to this respect are iPS cells, that can be generated from easily accessible sources through minimally invasive biopsies (i.e. skin fibroblasts) or a simple blood test (i.e. peripheral blood cells) [54].

Several complications also arise during the process that turns the starting cell source into the final product, ready for transplantation into the tissue of need. Genomic aberrations may appear due to extensive in vitro culture [58]. Indeed, the length of in vitro protocols required to achieve sufficient numbers of ready-to-use cells is also a cause of concern when therapeutic efficacy depends on their prompt administration after the insult [59]. In addition, such protocols are frequently complicated recipes that require finely tuned exposure to growth factors, xenobiotics and other substances in order to achieve the desired cell phenotype. Designing such recipes and optimising timing and dosage of exposure to specific cues is a daunting task, and substitutions are commonly needed when the presence of specific molecules in the culture is not considered safe for later human transplantation. Finally, even if the optimal cell product can be obtained in the laboratory, poor engraftment is often to blame in the discrete therapeutic efficacy achieved to date by cell replacement therapies [58, 60].

Direct generation of pluripotent or pluripotent-like intermediates in situ could bypass all limitations listed above, since no donor cell isolation, nor in vitro culture and manipulation, are required. Reprogramming is also reported to occur promptly in vivo after the administration of OSKM factors, without the need to co-administer other substances or adjuvants [2, 3]. In vivo, re-differentiation is also thought to take advantage of pro-differentiation signals naturally present in the host's tissue microenvironment, without the need to optimise complicated protocols to obtain specific cell types. In fact, cells differentiated within living tissues have been reported to achieve a more mature phenotype than those differentiated in the culture dish, which mainly attain a phenotype closer to embryonic or progenitor stages [29, 36]. Finally, chances of graft rejection are believed to be diminished since in vivo reprogrammed cells originate from the host's own organism. Indeed, reprogrammed cells have been seen to successfully re-integrate in the tissue and accomplish their physiological function upon re-differentiation in various studies [8, 20].

6.5 Needs on the Road Towards Clinical Translation of In Vivo Reprogramming Towards Pluripotency

While the first pre-clinical studies support the potential of in vivo reprogramming via OSKM overexpression to enhance tissue regeneration, various obstacles that this technology will need to overcome before it may turn into a clinical reality have also been made apparent. Key issues among them are those related to the fate of in vivo reprogrammed cells and to the efficient, yet safe, in vivo delivery of reprograming factors.

6.5.1 Transient OSKM Expression for Teratoma-Free In Vivo Reprogramming

Fear to the generation of teratomas due to uncontrolled proliferation and disorganised re-differentiation of in vivo reprogrammed cells has slowed down the pace of research on the therapeutic applications of in vivo OSKM induction.

Indeed some, but not all, of the studies in which OSKM factors were overexpressed in the living organism reported the generation of tumours within reprogrammed tissues, of which a vast majority were classified as teratomas based on the presence of tissue structures representative of all three germ layers [4, 8, 20, 27, 61, 62]. Others have however demonstrated complete absence of tumorigenesis even for extended periods of time after reprogramming [2, 3, 19, 21]. Table 6.2 compiles all studies on in vivo OSKM overexpression published to date, indicating the induction protocol of choice and the appearance or not or teratomas.

While such studies share similarities and differences in the way that OSKM factors are induced, the duration of their expression has been identified as the main determinant in the fate of in vivo reprogrammed cells and consequently in the appearance or not of teratomas [63]. The first study to report the development of OSKM-triggered teratomas relied on systemic (i.e. oral) doxycycline administration to induce pluripotency in reprogrammable mice — with OSKM transgenes inserted in the genome under the control of a doxycycline-inducible promoter — and already suggested the relevance of the temporal extent of OSKM expression in such an aberrant outcome. Administration of 0.2 mg/ml of the drug in the drinking water for a period of 2.5 weeks caused higher incidence of teratoma formation than a 5-times higher dose (1 mg/ml) that was withdrawn after 1 week. Teratomas were also found to develop faster with the longer induction scheme, and the survival of the animals was shortened compared to the higher — but shorter — dose [4].

Use of integrating viral vectors that sustain transgene expression for prolonged periods of time (i.e. retroviral vectors) to deliver OSKM also led to the development of teratomas within reprogrammed tissues [20]. Therefore, teratoma formation upon in vivo reprogramming is not limited to the use of genetically engineered reprogrammable mice.

Further studies have covered a wider range of induction intervals, always thanks to doxycycline-inducible OSKM expression, and confirmed the direct relationship between time of OSKM expression and incidence of teratomas. Remarkably, many of the animals fed with the drug for less than 5 days did not develop permanent dysplastic growth lesions or teratomas. Even when OSKM expression was maintained for up to 7 days, some of the cells reprogrammed to a de-differentiated and proliferative state where able to re-differentiate into a mature phenotype that successfully integrated in the tissue recapitulating its physiological function. An example of such event was reported in the pancreas of reprogrammable mice, where transiently reprogrammed cells expressed insulin after re-differentiation [8].

Nevertheless, strategies that achieve teratoma-free reprogramming rely on even more transient induction schemes. For example, delivery of OSKM factors in

Table 6.2 Studies on in vivo OSKM overexpression (updated May 2017)

Species	OSKM overexpression	Administration scheme	Target tissue	Teratoma formation	Reference
Tadpole	pDNA (OSK)	Single i.m. administration	Tail muscle	No	[2]
Mouse	pDNA (OSKM)	Single HTV administration	Liver	No	[3]
	Reprogrammable mouse	0.2 mg/ml Dox, 2.5 weeks or 1 mg/ml Dox, 1 week	Ubiquitous	Yes	[4]
	Dox in drinking water				
	Reprogrammable mouse	2 mg/ml Dox, 3-9 days	Ubiquitous	Yes[a]	[8]
	Dox in drinking water				
	Retroviral vectors (OSKM)	Single intracranial injection	Brain cortex	Yes	[20]
	Reprogrammable mouse	0.2 mg/ml Dox, 8 days	Ubiquitous	Yes	[61]
	Dox in drinking water				
	Reprogrammable mouse	1 mg/ml Dox, (2 days + 5 day withdrawal) 35 cycles.	Ubiquitous	No	[19]
	Dox in drinking water				
	pDNA (OSKM)	Single i.m. administration	Gastrocnemius muscle	No	[21]
	Reprogrammable mouse	0.2 mg/ml Dox, 7 days	Ubiquitous	Yes	[27]
	Dox in drinking water				
	Reprogrammable mouse	0.2 mg/ml Dox, 2.5 weeks	Ubiquitous	Yes	[62]
	Dox in drinking water				

This table compiles all studies on in vivo OSKM overexpression published before May 2017, including the species, tissue target, delivery method and appearance or not of teratomas

[a]Not all mice in the study developed teratomas, as highlighted in the text, which was strongly influenced by the duration of OSKM expression

plasmid DNA (pDNA) backbones that remain as episomes. As in vivo reprogrammed cells proliferate actively during the earliest phases of reprogramming, the episome is diluted with cell division and OSKM expression decays rapidly over time [2, 3, 21]. A different strategy established a short but cyclic OSKM induction protocol, again based on the doxycycline-inducible system, whereby the drug was administered for 2 days followed by 5-day withdrawal. Interestingly, this approach has proved to escape tumorigenesis for at least 35 repeats of the cycle [19].

Indeed, different OSKM induction protocols seem to have distinct effects on the extent of de-differentiation acquired by in vivo reprogrammed cells. In Abad et al.'s study, whereby OSKM expression was sustained over extended periods of time,

in vivo reprogrammed cells acquired totipotency features—a more primitive and plastic status than that of ESCs—and proved able to contribute to extraembryonic tissues [4]. On the opposite scenario, the very transient but cyclic induction protocol designed by Ocampo et al. induced sufficient epigenetic remodelling to erase several hallmarks of ageing and "rejuvenate" aged cells but did not lead to their complete de-differentiation, nor to the acquisition of pluripotency features [19].

While the extent of reprogramming and de-differentiation required to induce efficient regeneration (and whether that would be the same in different tissues and injury scenarios) is still not entirely understood, it is clear that transient OSKM expression is an absolute requirement to ensure safe, teratoma-free in vivo reprogramming that holds potential for clinical translation [63].

6.5.2 Efficient, Targeted and Safe Vectors for OSKM Delivery

Many of the studies exploring the concept of in vivo reprogramming to pluripotency to date have relied on the use of "reprogrammable" mice that include OSKM reprogramming factors integrated in their genome (Table 6.2). Such model bypasses complications linked to in vivo gene delivery, ensures high reprogramming efficiency and is unquestionably useful in mechanistic and proof-of-principle studies [64, 65]. However, it is unable to provide clinical relevance given the nature of its genetic modification. The search for appropriate gene delivery vectors that allow clinical translation of in vivo reprogramming via OSKM overexpression is therefore a priority. While it is difficult to make general assumptions (i.e. the design of the vectors may be greatly influenced by the specific requirements of the disease to be tackled through in vivo reprogramming), some common features will need to be considered to ensure efficient, yet safe, reprogramming.

Based on the reported direct relationship between the duration of OSKM expression and the development of teratomas, the main priority should be to identify a vector able to provide transient expression of such factors that is yet sufficient to translate into functional regeneration. Integrating vectors should therefore be ruled out from the list, unless they are accompanied by excisable or silencing mechanisms [63]. Episomal non-viral vectors have so far provided the most encouraging results to this respect [2, 3, 21]. However, experience gathered from the gene therapy field reminds us that the promise of such systems at the pre-clinical level should not be assumed at the clinical setup [66–68].

Targeting specific cell populations may also be a requirement to control the effects of in vivo reprogramming. To date, retroviral vectors have been used to limit OSKM expression to dividing cells, but transgene integration and sustained reprogramming preclude clinical translation [20]. Use of non-integrating vectors with cell specific promoters may offer an alternative to ensure targeting without compromising the safety of the approach.

Overall, the emphasis in vector design for in vivo reprogramming in tissue regeneration should be placed in finding the appropriate balance between safety,

avoiding prolonged and/or ubiquitous expression of reprogramming factors, and efficacy, through the generation of sufficient reprogrammed cells to replenish the lost tissue.

6.6 Conclusions and Future Challenges

Although studies on in vivo cell reprogramming via direct OSKM overexpression are still scarce, the potential of this strategy to contribute to tissue rejuvenation and regeneration has already been confirmed by preliminary but sound studies that involve different tissues and injury models. The extent of reprogramming required for efficient regeneration, either to full pluripotency or via partial reprogramming accompanied by proliferation, remains to be determined and will likely depend on the nature of the specific condition to be tackled. However, some requirements needed to translate in vivo reprogramming towards pluripotency into a viable clinical approach have already been established. Transient OSKM expression is key to avoid tumorigenesis. Therefore, strategies involving sustained expression of reprogramming factors (i.e. sustained pluripotency) will not develop into clinically relevant approaches and should solely be considered as research tools to investigate the mechanisms behind the pluripotent conversion. Special efforts should instead be placed in designing appropriate delivery vectors that ensure efficient yet transient OSKM expression.

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