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Cell Cycle News & Views

Control of RNA polymerases I and III by the TOR signaling pathway

Comment on: Wei Y, et al. Cell Cycle 2009; 8:4085-90.

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Ribosome biosynthesis in eukaryotes is a highly coordinated process requiring a concerted activity of three nuclear RNA polymerases (RNA Pol I–III). In yeast RNA synthesis by Pol I and Pol III represents more than 80% of all nuclear transcription.[1] RNA Pol I is dedicated to the synthesis of 35S pre-rRNA which is subsequently processed to ribosomal 25S, 18S and 5.8S rRNA. tRNAs, 5S rRNA and several other small noncoding RNAs are synthesized by Pol III. Because of the high energy cost of Pol I and Pol III activity, coordination of their activities with environmental growth conditions provides a powerful selective advantage. Much of this regulation is achieved by the TORC1 complex with the evolutionarily conserved TOR kinase as the catalytic subunit. TORC1 activity is inhibited by the macrolide drug rapamycin which binds to the complex and suppresses its interaction with target substrates. Yeast TORC1 shuttles dynamically between the cytoplasm and nucleus and when present in the nucleolus, it binds to rDNA chromatin, 35S as well as 5S genes.[2] Significantly, although TORC1 does not bind to tRNA genes, it modulates tRNA transcription indirectly by phosphorylation of a Pol III repressor, Maf1.[2] Maf1, first identified in yeast 3, is an evolutionarily conserved general negative regulator of Pol III. Upon rapamycin treatment, Maf1 is imported to the nucleus,[4,5] and is detectable in the nucleolus.[2] TORC1 interacts with Maf1 and phosphorylates Maf1 at the rDNA loci, in this way regulating its translocation from the nucleolus to the nucleoplasm.[2] However, yeast Maf1 contains nearly 30 potential phosphorylation sites specific for several different kinases beside TOR (data according to Swiss-Prot PROSITE). Moreover, phosphorylation was recognized earlier as the main way of inactivating Maf1 in favorable growth conditions, by preventing its interaction with Pol III.[4,5] Additionally, phosphorylation acts both to relocate the nuclear pool of Maf1 to the cytoplasm[6] and to prevent the import of cytoplasmic Maf1 to the nucleus.[7] The paper by Wei and Zheng, published in this issue of *Cell Cycle* describes Sch9 as a Maf1 kinase and proposes a mechanism by which TORC1 and Sch9 jointly control Pol I and Pol III in yeast[8] (Figure 1).

Sch9 is a member of the AGC kinase family that mediates TORC1 regulation of ribosomal protein genes transcription by Pol II.[9] Yeast Sch9 is phosphorylated at multiple sites by TORC1, which is required for Sch9 catalytic activity.[9] Replacing these residues with acidic amino acids yields a protein variant termed Sch9(2DE), which is active even in the absence of an upstream signal from TORC1. Since Pols are co-regulated by TORC1, it is not surprising that also Sch9 is involved in Pol I and Pol III control. This is likely to constitute a highly efficient mechanism for coordination of protein biosynthesis. Maf1, identified by Wei and Zheng as an Sch9 target, functions in this mechanism as a mediator of the nutrient signal to Pol III.[8] Sch9 interacts with Maf1 and phosphorylates Maf1 in a rapamycin-dependent manner. Moreover, in the Sch9(2DE) mutant Maf1 phosphorylation is independent of rapamycin treatment, confirming that Sch9 mediates TORC1 signaling. Altogether, the data of Zheng's group show that TORC1 controls Maf1 not only indirectly in the nucleolus but also directly, via Sch9 in the nucleoplasm. To pinpoint the role of Sch9 in this regulation, Wei and Zheng showed that combined mutations inactivating Sch9-dependent phosphorylation sites on Maf1 are sufficient to cause Maf1 entry to the nucleus, essentially the same phenotype as that caused by Sch9 inactivation. Sch9 affects Pol III activity in an obvious way: tRNA synthesis was increased in the hyperactive mutant Sch9(2DE) and markedly decreased in a strain lacking Sch9. Importantly, the basal Pol III transcription that occurs in the absence of Sch9 was still sensitive to rapamycin, thus validating the previously described Sch9- independent effect of TORC1 on Pol III activity.[2,10]

The point of TORC1-mediated Pol III control is the regulation of nuclear-nucleolar Maf1 dynamics. Nucleolar localization of Maf1, crucial for Maf1 activation, is not affected by Sch9 restricting this control to TORC1 itself. Is then the role of Sch9 phosphorylation of Maf1 limited to promoting its cytoplasmic location? This seems to be an underestimation since cytoplasmic-nuclear transport of Maf1 is known to be dispensable for Pol III regulation.[6,7] Moreover, a recent report by Huber et al. (2009) shows that besides affecting its nuclear transport, Maf1 phosphorylation by Sch9 regulates its interaction with Pol III.[11] Mutations of Maf1 that mimic phosphorylation by Sch9 prevent its association with Pol III while inactivation of all Sch9 target sites in Maf1 promotes Maf1-Pol III association. How it happens, assuming the effect of Sch9 on Maf1 is restricted to nucleoplasm and Maf1 final destination is the nucleolus, remains to be elucidated. Nevertheless, inactivation of Sch9 does not promote interaction of Maf1 with Pol III.[11] Additionally, neither Maf1 mutants preventing nor these mimicking Maf1 phosphorylation by Sch9 did not affect the rapamycin–mediated repression of Pol III,[11] providing a final argument for the conclusion of Wei and Zheng that phosphorylation of Maf1 by Sch9 is not crucial for Pol III regulation in response to nutrient.

The balanced production of 5S and 35S rRNA requires coordinated regulation of Pol III and Pol I. TORC1 was proposed to control Pol I via recruitment of the essential Rrn3 initiation factor.[12] Sch9 is also involved in rapamycin-sensitive regulation of Pol I transcription,[8,11] although the mechanism is unknown. Obviously, multiple levels of control are involved in the balancing of the numerous ribosomal components.

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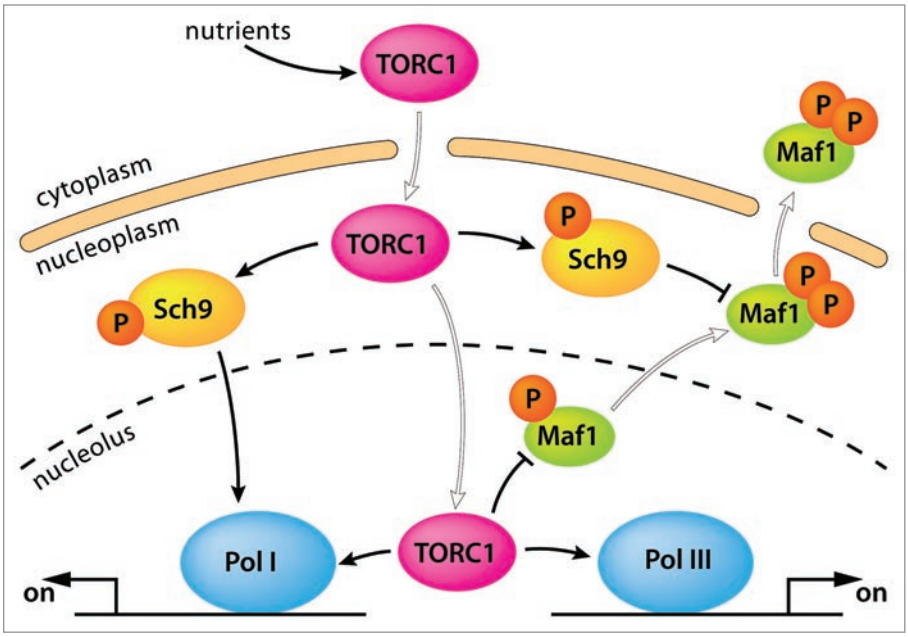

Figure 1. Sch9 partially mediates TORC1 signaling to activate Pol I and Pol III transcription by nutrients. In the presence of nutrients TORC1 promotes Pol I and Pol III transcription in the nucleolus and phosphorylates Maf1 preventing its negative effect on Pol III. Additionally, TORC1 activates Sch9 and uses Sch9 to phosphorylate Maf1 in the nucleus and promote its export to the cytoplasm. Bold lines, activation/repression; empty lines, changes in localization).

New tricks from an old drug: A role for quinacrine in anti-cancer therapy?

Comment on: Neznanov N, et al. Cell Cycle 2009; 8:3960-70.

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When cells are exposed to proteotoxic stress, they depend on the activation of several cytoprotective cellular response mechanisms for survival. To protect against this stress, cells induce the heat shock response (HSR), an ancient and highly conserved genome wide transcriptional program, by triggering the activation of heat shock factor 1 (HSF1), the master transcription factor that regulates inducible heat shock gene expression and the synthesis of additional chaperones. HSF1 coordinates induction of the HSR to refold and repair damaged proteins, preserve protein function and metabolism, in order to restore cellular homeostasis and maintain cell survival under stress.[1]

Since cancer cells undergo extreme proteotoxic stress, upregulation of the heat-shock response is essential to alleviate the negative effects of increased protein misfolding and turnover, competition among proteins for access to chaperones, and proteasomal stress. As such, tumors exhibit elevated levels of several heat shock proteins (HSPs), including HSP90,[2] suggesting that HSP overexpression may contribute to aberrant tumor survival. Therefore, HSF1 is not only constitutively active but also orchestrates a broad network of key cellular processes that includes protein synthesis, glucose metabolism, cell proliferation and survival, to enhance oncogenic transformation. Indeed, HSF1 has been shown to be a potent modifier of tumorigenesis, facilitating in the initiation and maintenance of various cancers.[3] Although HSF1 is neither a classical oncogene nor tumor suppressor, cancer cells can become dependent on HSF1 to modulate an entire network of cellular functions that enable tumorigenesis referred to as "non-oncogene addiction."[4] While oncogenes make attractive targets for the development of cancer therapeutics (e.g., tyrosine kinase inhibitor imatinib), recent drug development efforts have been devoted to the growing list of non-oncogenes (e.g., proteasome inhibitor bortezomib) that may also be exploited as potential cancer drug targets and such is the case for the discovery of HSF1 inhibitors.[5]

In *Cell Cycle* Volume 8, Issue 23, Neznanov and colleagues show that the anti-malarial drug quinacrine can suppress HSF1-mediated heat shock response induced either by the proteasome inhibitor MG132, hsp90 inhibitor 17-DMAG, or hyperthermia. The reduced HSF1-dependent transcription occurs at a point downstream of its cytoplasmic activation, nuclear translocation, and DNA binding, suggesting that quinacrine uses a mechanism to block transcription initiation that requires its own nuclear localization, similar to its inhibitory effect on NF-κB-mediated transcription.[6] Moreover, the effects of quinacrine on inducible transcription appear selective since the drug stimulates p53-mediated transcription6 and can inhibit the prosurvival AKT/mammalian target of rapamycin (mTOR) pathway that lies downstream of PI3K.[7] The functional interplay among the PI3K/AKT/mTOR, p53, NF-κB and now HSR pathways make quinacrine an even more attractive anticancer drug candidate since it appears to simultaneously and selectively target these multiple pathways.

The remarkable finding in the study lies in the combination treatment of quinacrine with proteotoxic stress inducers (e.g., proteasome and HSP90 inhibitors). Because targeting HSP90 using inhibitors such as 17-DMAG has shown

limited therapeutic success in the past, the addition of quinacrine in this study actually enhances the cytotoxicity of 17-DMAG, augmenting both drugs' efficacy. While the mechanism of enhanced cytotoxicity remains to be elucidated, a recent study shows that p53 functional status is a likely predictor of the sensitivity of tumors to 17-DMAG and that the administration of 17-DMAG both retards tumor growth and engages p53-dependent cell death in vivo in murine medulloblastoma.[8] It will therefore be interesting to determine the degree to which quinacrine can further enhance the cytotoxicity of 17-DMAG in this cancer model since quinacrine also stimulates the p53 pathway and is known to cross the blood-brain barrier.

Taken together, these studies demonstrate that targeting the heat shock response, specifically HSF1, may facilitate the use of proteasome and HSP90 inhibitors for cancer treatment and opens up a unique avenue to explore potential treatment strategies involving these two classes of inhibitors within the HSR pathway. In fact, a screen of 20,000 small molecules for compounds that inhibit HSP induction identified two analogs of the general translational inhibitor dehydroemetine that can sensitize cancer cells to the effects of proteasome and HSP90 inbiitors.[9] Most recently, using a high-content target-based screening assay for identifying small molecule functional inhibitors of HSF1, Au et al. has discovered a compound that inhibits HSF1 granule formation and inhibits HSF1 phosphorylation.[10] Whether investigators are involved in the discovery of novel selective HSF1 inhibitors or uncovering a previously unknown HSF1-mediated mechanism of action of an existing drug, future studies are warranted in pre-clinical evaluation of their anticancer activity and potential therapeutic combinations with other classes of drugs (e.g., kinase inhibitors).

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DNA damage response in the absence of DNA lesions continued…

Comment on: Pospelova TV, et al. Cell Cycle 2009; 8:4112-18.

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Breaks in DNA are caused by exogenous sources such as ionizing radiation (IR), radiomimetic drugs and by endogenous sources such as free radicals or replication fork collapse at single-strand lesions during S phase.[1] Double Strand Breaks (DSBs) are amongst the most serious DNA lesions because they are difficult to repair, due to the lack of an undamaged complementary strand. Such breaks are usually repaired through homologous recombination (HR) and non-homologous end-joining (NHEJ). HR takes place in the late S-G_2 phases, when DNA is replicated, while NHEJ happens mostly in the G1 phase of the cell cycle.[1] The activation of the DNA damage response leads to cell cycle arrest until repair has taken place. The players of the DNA damage response are the components of the early DNA damage sensor complex MRN (MRE11/Rad50/NBS1), the transducer proteins MDC1 and 53BP1, the PI3 kinases ATM/DNAPK/ATR, which phosphorylate the histone variant H2AX, and the downstream effectors including the Chk1 and Chk2 cell cycle kinases[1] Unsuccessful DNA repair and accumulation of DNA damage can lead to mutations and chromosomal abnormalities, which are hallmarks of cancer.[1] DNA repair pathways also have other essential roles in longevity since insufficient repair mechanisms can lead to senescence, as shown by studies performed on mice defective for DDR proteins[2]

Until very recently, the DNA damage response has been strictly associated with the induction of breaks to the DNA. This dogma has been lately challenged by several observations in mammalian and yeast cells. In this issue of *Cell Cycle*, Pospelova and colleagues report an additional case of a "pseudo DNA damage response," which is manifested during senescence. They show that upon induction of senescence by activating p21 or p16, specific DDR markers are activated in the absence of detectable DNA breaks. More specifically, they observe robust formation of γ-H2AX foci and uniform nuclear signal of phosphorylated ATM. Nevertheless, the observed DDR has several differences with the classical DDR activated by DNA damage. First, not all of the key players of the DDR appear to be implicated in this new pathway. While γ-H2AX foci are formed, 53BP1 foci are totally absent and phosphorylated ATM has a uniform nuclear staining that doesn't colocalize with the γ-H2AX foci. Second, the temporal kinetics of this DDR is slower than the one activated by DNA lesions.

There is increasing evidence that DDR can be mounted in the absence of DNA lesions.[1] Immobilization of repair proteins on chromatin to mimic foci formation both in yeast and mammalian cells was shown to be sufficient to activate markers of DDR.[3,4] Moreover, constitutive activation of ATR by overexpression of small domain of the ATR-stimulating TopBP1 protein leads to DNA damage-independent activation of DDR and subsequent cell cycle arrest and senescence.[5] Moreover, γ-H2AX is activated during mitosis where chromosomes are condensed.[6] Interestingly, the γ-H2AX signal in mitotic chromosomes is devoid of 53BP1. This observation is in line with the pseudo DDR activation shown by Pospelova et al after treatment with the histone deacetylase (HDAC) inhibitor Nabutyrate to induce senescence, as well as the observed lack of colocalization between γ-H2AX and 53BP1 in early mouse embryo in the absence of DNA damage.[7] These observations raise the possibility that the DDR machinery senses not lesions per se but also changes in chromatin structure. Consistent with this notion, ATM is activated in response to changes in chromatin structure upon exposure of cells to the HDAC inhibitor TSA.[8]

Although a classical DDR has so far been proposed to induce senescence,[9] Pospelova et al demonstrate for the first time a DD-like response that is the consequence, not the cause, of senescence and they suggest that pseudo DDR is acting as a mechanism of perpetuating the senescence phenotype and protecting cells

from reentering to the cell cycle, which can lead to cellular abnormalities.

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Defining the ERAD connection: Assembly required

Comment on: Otero JH, et al. Cell Cycle 2010; 9:In press.

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The proper storage and transfer of biological information is of paramount importance to all living cells, and requires the services of numerous quality control systems to ensure operational fidelity.[1] Because these systems constitute a series of cellular "checkpoints," many have begun to question whether (and how) they might be coordinately regulated. This question is of particular interest to cancer cell biologists because numerous quality control systems are compromised during the process of carcinogenesis. In fact, the meaningful discovery of biomarkers to used as reliable diagnostic tools or prognostic indicators might depend on our ability to ultimately decipher how these interconnections are regulated, rather than to merely monitor changes in the intracellular concentrations of individual quality control components.

In a forthcoming issue of *Cell Cycle* (Volume 9, Issue 1), Chang and colleagues continue their use of fission yeast to reveal that a protein complex known to facilitate the proper segregation of chromosomes during mitosis plays an additional role in regulating the quality of proteins that traverse the early secretory pathway. The authors had already identified the participation of these proteins in the proteasomal destruction of nucleo-cytoplasmic proteins (involved in chromosome segregation), as well as part of a supercomplex that recruits proteasomes to the translation machinery as a means to cotranslationally degrade proteins unable to attain conformational maturation.[2] The importance of the new study stems from their ability to begin to define the physical connections that establish this additional link to protein quality control in the secretory pathway where the events of translation and quality control are often temporally and spatially separated.

As a rule, proteins unable to correctly fold in the endoplasmic reticulum (the first compartment of the secretory pathway) are selectively dislocated back into the cytosol in response to the covalent addition of ubiquitin which also promotes proteasomal degradation.[3] The entire process, consisting of sequential substrate selection and dislocation events has been designated 'endoplasmic associated degradation' (ERAD).[3] Cdc48, the yeast homolog of mammalian p97, is known to transfer escort ubiquitinated ERAD substrates to proteasomes at the ER membrane.[4] The present study shows that Cdc48's contribution is made possible through its physical interaction with Moe1 which is itself bound to Yin6, the latter of which is directly bound to proteasomes. Importantly, Moe1 and Yin6 are the fission yeast orthologs of two important eukaryote translation initiation factor 3 subunits, demonstrating the functional link to protein translation. Genetic deletion of the complex is not lethal, implying that it is not absolutely required and therefore either functions to enhance the global efficiency of ERAD or is responsible for the eliminating only a subset of ER-derived proteins.

These multiple capacities might stem from the abilities of Cdc48 and the Yin6-Moe1 complex to individually influence additional processes in the cell, including membrane fusion and intracellular protein transport. As such, the coordinate regulation might involve the dynamic assembly of an even larger molecular machine that is shuttled to distinct parts of the cell either in response to covalent modifications or through the utilization of interchangeable adaptors. In fact, this notion is in agreement with the fact that none of these proteins are known to directly interact with biological membranes. Additional studies are required to determine whether a "scaffold-dependent shuttling" model operates in fission yeast or in mammalian cells.

Importantly, the altered expression of these proteins is often associated with cancer. Therefore, its role in chromosome segregation could account for the aneuploid phenotype often associated with cancer. In addition, a prolonged ER stress response that involves ERAD[5] is known to induce apoptosis.[6] Therefore, a diminished concentration of the protein might actually prevent apoptotic cell death, providing cancerous cells with a competitive growth advantage. In fact, the idea fits well with the evolving paradigm that cancer progression results from the mis-regulation of numerous mechanisms, including an imbalance in the levels of pro-apoptotic and anti-apoptotic factors.

Finally, it is not surprising that cellular functions might be under the control of dynamic molecular machines that consist of interchangeable parts (Figure 1). This arrangement would allow for the coordinate linkage of checkpoints that must operate throughout living cells. The acquired knowledge generated from this, and additional, studies should help to illuminate the fundamental mechanisms by which this series of critical checkpoints are functionally linked, and eventually demonstrate whether any apparent mis-regulation associated with cancer is amenable to mechanism-based therapeutic intervention.

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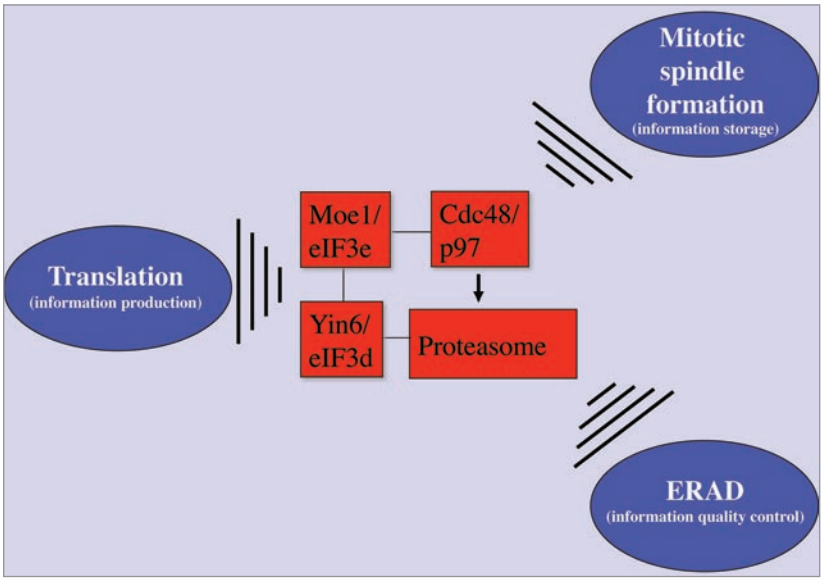

Figure 1. A protein complex can apparently regulate mitotic spindle formation, protein translation, and proteasomal degradation, possibly by employing interchangeable parts and/or through a scaffold-dependent shuttling mechanism. Moe1 and Yin6 are the fission yeast homologs for the eukaryote translation initiation factors eIF3e and eIF3d, respectively, that provides a molecular scaffold that allows Cdc48 to escort ERAD substrates for degradation by cytoplasmic proteasomes.

Transdifferentiation of endogenous cells: Cell therapy without the cells

Comment on: Bukovsky A. Cell Cycle 2009; 8:4079-84.

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Regenerative medicine is the replacement of damaged or diseased cells with new healthy functional cells, usually from an exogenouse source. Although embryonic stem cells play a central role in cell based therapy research the best example is the use of hematopoietic stem cells for bone marrow transplant to treat blood malignancies.[1]

Decades of research have produced promises and revealed pitfalls of cell based therapy.[2] There are a number of hurdles that need to be addressed in order for stem cells to be considered for therapeutic use, such as safety, sufficient cell numbers, accessibility, availability when required, efficient mode of delivery to the target organs, high engraftment and differentiation rate and a functional improvement after transplantation. Some laboratories have chosen to address these hurdles by taking advantage of endogenous stem cells instead of using an exogenous cell source. By inducing the patient's own cells, in situ, to act as a replacement cell source, the problems of tissue matching, availability and delivery are eliminated leaving only the issue concerning efficiency of differentiation and return of tissue function. An example of this has been demonstrated by the stimulation of endogenous cells in spinal cord injury models.[3]

However the use of endogenous stem cells also has its limitations. The tissue damage could be so extensive that the endogenous stem cells or the cell niche could be damaged or even absent. For example, in the case of type 2 diabetes there is evidence that the endogenous endothelial progenitor cells are damaged and incapable of repairing damaged blood vessels.[4] To circumvent this problem, in the cases similar to the situation found with diabetes, we can come full circle and suggest the use of exogenous stem cells or use a novel approach which is to take advantage of trans-differentiation and induce unrelated cells in the vicinity of the injured area to contribute to tissue repair.[5] Although trans-differentiation is a poorly understood process the ability of the mature cells to give rise to cells of a different type has been well documented. The ability of cells to trans-differentiate has been demonstrated in multiple systems although whether the event is due to trans-differentiation6 or reprogramming to an early embryonic state followed by redifferentiation to a new mature cell type, remains unclear.[7,8]

Historically, the ability of cells to transdifferentiate or to be reprogrammed went against embryo and developmental biology paradigms that described development as a stage specific, linear progression which lead to a terminally differentiated state. Regeneration in amphibians and wound healing in mammals suggested that there were exceptions to the rule. In the last decade there have been multiple reports suggesting trans-differentiation of mature cell types[9] but in some cases these reports were also challenged.[10] After almost a decade of controversy it was reported that the introduction of just four genes were capable of converting a skin fibroblast to a cell identical to pluripotent embryonic stem cells.[8] This work and subsequent publications clearly demonstrate the plasticity of cells, opening the door to studies such as the one described here.

Dr. Bukovsky previously demonstrated that a combination of sex steroids such as estradial, testosterone and progesterone have the ability to trans-differentiate ovarian epithelial cells to neural stem cells.[11] In their recent publication they have demonstrated that smooth muscle cells in the vasculature can also be induced to trans-differentiate into neural cells using the same combination of sex steroids (Bukovsky, this journal). Sex steroids are not normally used to induce the differentiation of stem cells or the trans-differentiation of mature cells as the majority of reports have used transgenes or growth factors. Although neural cells appeared infrequently in the cultures their appearance increased if the cells were exposed to combinations of estradial, progesterone and testosterone, however, individually these factors are ineffective. Current methods using transgenes, recombinant growth factors or chemicals to induce endogenous cells to repair damaged

tissue may not be suitable for the clinic but sex steroids are good candidates as their main advantage is that they are normally present and are able to cross the blood-brain barrier leading to the stimulation of endogenous cells in the brain and spinal cord. Although stem cell based therapy holds much promise the issues of cell source, tissue matching and cell delivery are major hurdles that need to be overcome. This can be solved by the stimulation of endogenous cells localized to the area of damage. Furthermore not having to use exogenous cells or transgenes will fast track this technology through clinical trials.[12,13]

The investigation of sex steroids as inducers of trans-differentiation of smooth muscle cells associated with the vasculature into neural cells is novel and introduces an alternative method of regenerative medicine. Smooth muscle cells are abundant throughout the vasculature and stimulation of these endogenous cells to produce neurons or neural stem cells in an area of neural damage could lead to repair and the return of function without the requirement for cell transplantation.

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