Activity of p53 in human amniotic fluid stem cells increases their potentiality as a candidate for stem cell therapy

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Abstract:

The potential use of stem cells as a therapeutic treatment for many neurological disorders, such as stroke, has spiked an interest in their properties. Due to limitations of the present-day treatments, regenerative and protective therapies could prove very beneficial if a safe and effective treatment is identified. Using human amniotic fluid stem (hAFS) cells could theoretically provide both neuroprotective and regenerative properties to patients, and knowledge of p53's activity and function could be a key component in understanding the behavior and characteristics of these stem cells to harness their full potential. Many recent studies on p53 have provided new and valuable information that could give rise to new ideas for treatment options. More specifically, p53's activity inside hAFS cells lead them closer to becoming a potential therapeutic stem cell. Other neuroprotective treatments, such as hyperoxia and hypoxia sessions, are showing positive results. In combination, these data are helping to get closer to an effective treatment for neurological disorders.

Keywords:

Cerebral ischemia, human amniotic fluid stem cells, p53, regenerative medicine, stem cell therapy

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Introduction: p53 Activity and Human Amniotic Fluid Stem Cells

In a study done on p53 inside amniotic fluid stem cells, it was found that undifferentiated human amniotic fluid stem (hAFS) cells express p53 at lower levels than cancerous cells. The p53 protein is found primarily in the nucleus of the hAFS cells. The anti-proliferative activity of p53 was limited. p53 regulates two target genes, namely *igf2*, a maternal imprinted gene and *c-jun*, a proto-oncogene. When DNA is damaged, the amount of p53 increases and consequently so does the activation of its target genes. Differentiation of amniotic fluid stem cells toward the neural lineage induces p53.[1] The hAFS cell line used was tested for several intracellular and surface markers. This was tested to confirm that the hAFS cells are in a middle state of pluripotency between that of ES cells and lineage-restricted adult progenitor cells.[2] hAFS cells showed the expression of various mesenchymal markers, several-related surface adhesion molecules, and stemness markers; however, they did not show hematopoietic surface markers.[1]

p53 Location and Function in Human Amniotic Fluid Stem Cells

It was determined that the p53 protein was localized in the nucleus.[1] However, p53's abundance was heterogeneous with some cells expressing high concentration but mostly low and variable levels of expression in the early and late passages, but data revealed that expression of the p53 protein remained the same with increased passage numbers. Due to the restrictions on using

embryonic stem cells (ES), p53 abundance was compared with different tumor cells because they have relatively similar amounts to human ES cells.[1] Data concluded that hAFS cells had a much lower abundance of p53.

Previous findings demonstrating that p53's antiproliferative activity is compromised in murine ES cells was tested in hAFS cells.[3] p53 was downregulated, and cell number was monitored. Only a slight difference between control cells and downregulated cells was seen. Results were very similar to those in the murine ES cell study and indicated that the anti-proliferative activity of p53 in hAFS cells was compromised.[1]

*igf*2 and *c-jun* Expressions are Regulated by p53 in Human Amniotic Fluid Stem Cells

p53 does not suppress the cell proliferation in unstressed hAFS cells.[1] Two noncanonical target genes which are induced by p53, *c-jun* and *igf*2 were measured. p53 was downregulated, and expression of genes was measured. *c-jun* expression was reduced, whereas *igf*2 expression was surprisingly increased. While the results of *c-jun* expression were congruent with previous findings, the *igf*2 results contradicted the results on ES cells. To further investigate, p53 was overexpressed but *igf*2 messenger RNA (mRNA) levels remained the same.[1]

Induction of p53 During Differentiation in Human Amniotic Fluid Stem Cells

Due to the previous findings that p53 is involved in differentiation in ES and adult stem cells,[4-6] it was investigated whether p53 had any contribution to differentiation in hAFS cells. hAFS cells were differentiated over 24 days and monitored closely. Days 17–24 had the highest expression of p53, and at the same time, these were the days when Nestin, MAP2, and β-tubulin III were expressed.[1] Next, to see if the differentiation of hAFS cells was a p53-dependent event, the transcriptional activity of p53 was blocked. Following this, nestin amounts were seen to be reduced, which indicated that differentiation was reduced.[1]

Human Amniotic Fluid Stem Cell DNA Damage Activates p53

After DNA damage, one of p53's jobs is to arrest the cell cycle and induce apoptosis.[7] p53 abundance and activity are increased in response to DNA damage.[8] It was found that p53 is important in the DNA damage response because of its activation of caspases and apoptosis.[9-12] Caspase 3 is responsible for cleaving the poly [ADP-ribose] polymerase (PARP) protein. Therefore, PARP cleavage during DNA damage response was monitored under the normal expression of p53 and when p53

was downregulated. It was shown that when p53 was downregulated, the increase in cleavage was less than that of when p53 is normally expressed, showing that p53 is actively involved in DNA damage response.[1]

Why p53?

Since the identification of p53, an essential transcription factor found in multicellular organisms, it has been at the center of cancer research due to its contributions to many cellular processes such as proliferation, senescence, differentiation, apoptosis, ferroptosis, DNA repair, metabolism, angiogenesis, and autophagy.[4,13-16] As a transcription factor, p53 primarily functions by activating transcription of target genes.[1] However, its ability to directly interact with proapoptotic and antiapoptotic proteins also gives it the potential to promote apoptosis.[17] Concurrent with its role in adult somatic cells, p53 seems to be involved with self-renewal and differentiation of ES cells as well as some adult stem cells. p53 also possesses the ability to negatively regulate and maintain quiescence of adult stem cells such as neural and hematopoietic cells.[18-20] hAFS cells, found in a median state between ES cell pluripotency and lineage-restricted adult progenitor cells, possess the p53 tumor suppressor gene.[1,21] hAFS cells also proliferate quickly as well as exhibit a wide differentiation range, including the ability to become hematopoietic, neurogenic, osteogenic, chondrogenic, adipogenic, renal, and hepatic lineages.[21-23] Alongside these promising attributes, during laboratory trial, when hAFS cells were transplanted into nude mice, they did not cause the formation of teratomas while ES cells did.[24] Although very promising in the potentiality of being a source of therapeutic stem cells, the activity of p53 in hAFS cells is not well known. Defects or loss in p53 function can have detrimental effects on genomic stability.[1] This article presents that p53 is active in hAFS cells and is found primarily in the nucleus. Under nonstressed conditions, p53's anti-proliferative activity is limited, however, becomes active in response to DNA damage. Furthermore, two genes are regulated by p53 in hAFS cells: *c-jun*, a proto-oncogene, and *igf*2, a gene important in cellular proliferation and development.[1]

Human Amniotic Fluid Stem Cells Could Be a Potential Therapeutic Stem Cell

Several lines of investigation were conducted to identify a potential cell type for therapeutic stem cell injections into humans. Recent findings have found that the once-promising candidate of ES cells, frequently generate mosaic alterations and that p53 is often mutated in human ES cell lines.[25,26]

The ideal stem cell candidate would have no ethical controversy, be easy to obtain, divide rapidly in culture,

and shows broad plasticity.[1] Along with fulfilling all these requirements, hAFS cells do not form tumors when transplanted into mice. ES cells, on the other hand, formed teratomas when transplanted into mice.[24,27] The function and activity of p53, an important tumor suppressor protein, must be identified before hAFS cells are used in therapy. Overall, hAFS cells show great potential to 1 day be used as a therapeutic stem cell.

When initially identified, the p53 protein was discovered to be localized in the nucleus of hAFS cells.[1] Consistent with these data, the results of a previous report locate p53 in the nucleus of murine ES cells.[3] Other previous reports about p53 mRNA concentration also stay congruous with the results that p53 protein levels remained relatively constant and did not change with increasing passage numbers.[28,29]

While wild-type p53 is an anti-proliferative protein, when mutated, it is commonly associated with tumor growth.[1] Therefore, the effect of p53 on the proliferation rate of hAFS cells was monitored. The results suggested no difference in proliferation capacity between control cells and cells where p53 was downregulated.[1] These data are consistent with the previous findings with murine ES cells where anti-proliferative activity of p53 was compromised.[3]

Regulation of two noncanonical target genes of p53, *c-jun*, and *igf2*, was measured in hAFS cells. These genes were also regulated by p53 in ES cells.[3] The result was that repression of *c-jun* by p53 in hAFS-matched previous data from ES cell research. However, *igf2* mRNA was repressed in ES cells, it was found that in hAFS cells, p53 induced *igf*2 mRNA levels.[3] *c-jun*, a proto-oncogene, achieves its growth-promoting function through heterodimerization with c-Fos, binding to AP-1 responsive elements in promoters of their target genes, and repression of tumor suppressor genes, namely p53, p21, and p16.[30] *c-jun* has also been shown to have the ability to directly bind to and repress the p53 promoter.[1]

igf2, a proto-oncogene involved in the development, is another target gene of p53 and is often overexpressed in tumors.[31,32] While no reduction in *igf2* mRNA was seen during overexpression of p53, the downregulation of p53 strongly induced *igf2* mRNA.[1] There is no clear understanding of why this inconsistency exists. Furthermore, the expression of *igf2* was seen in cells with a female karyotype, but not male karyotyped cells.[1] This could be due to that eventually in males, *igf2* expression is not required and that during deletion of the *igf2* gene, male cells are still viable. However, female cells are strongly dependent on *igf2*.[33]

As differentiation progressed in hAFS cells, p53 was strongly induced.[1] When transcription of p53 during differentiation was blocked, it resulted in decreased nestin amounts, exhibiting that p53 contributes to the differentiation of hAFS cells.[1] Surprisingly, p53's increase in abundance during differentiation contradicts previous studies in ES cells, where p53 abundance decreased as differentiation progressed.[16,34-36] p53 plays a role in the DNA damage response.[37] In hAFS cell experiments, p53 became activated and its levels increased, and target genes p21 and mdm2 were induced. Interestingly, it was found that in response to the DNA damage, the cleavage of PARP, a DNA repair protein, was a partly p53-dependent event.[1] No other DNA damage agents were tested, and further experimentation must be done to determine if this response is specific to some agents or universal among many.

Amniotic fluid contains cells derived from the fetus and amnion; there is a possibility of donor-to-donor heterogeneity that could influence proliferation rate, differentiation capability, and DNA damage response.[1] These experiments were conducted using a single donor hAFS cell line. Further experiments must be conducted with different donors to rule out any genetic factors that would influence the hAFS cell activity.

In summary, evidence suggests that p53 is active in hAFS cells. Differences in p53 activity between hAFS cells and ES cells have been indicated, leading to inferences that there is no generalized activity of p53 across stem cells.[1] This is also demonstrated by differences in p53 expression across different mesenchymal stem cell types.[38] While hAFS cells are of potential usage for stem cell therapy, heterogeneity must be further investigated to rule out any possibility of differences in the behavior of cells among donors.

The Further Investigation of the Role of p53 in Human Amniotic Fluid Stem Cells Could Lead to Pioneering Medical Advancements

Stroke is a leading cause of death and often results in long-term disability.[39] Developing safe and effective treatments poses a great challenge. With a few current treatment options, stroke researchers are identifying many possible therapies to better treat stroke patients. Currently, the only stroke treatment approved by the Food and Drug Administration is a thrombolytic drug or a tissue plasminogen activator.[39] While it has been demonstrated effective in dissolving clots, there is a massive time constraint due to the requirement of administering the drug within 4.5 h after a stroke.[39] Other treatments such as surgical thrombectomy or embolectomy are effective, but pose more risks with older patients.[39] Neuroprotectant treatments and regenerative therapies could also prove very beneficial as effective treatments for strokes. Stem cells could potentially

serve as both a regenerative and neuroprotective agent. However, stem cell treatments often lead to ethical controversy, and it is challenging to secure an ideal candidate.

hAFS cells have shown great potential to be utilized as therapeutic stem cells [Figure 1]. As an ideal candidate, hAFS cells display pluripotency in that they can differentiate into all three germ layers. Furthermore, there are minimal ethical issues surrounding the harvest and usage of hAFS cells. They are harvested during amniocentesis and provide a heterogeneous cell pool, including amniotic fluid-specific cells, fibroblastic cells, and epithelioid cells. Derived hAFS cells can become human amniotic mesenchymal stromal cells that serve as anti-inflammatory and anti-fibrotic agents effective in treating other neurological diseases.[40] hAFS cells show great efficacy in treating neurological conditions such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, spinal cord injury, and more.[40] A study was done to investigate the regenerative properties of hAFS cells after an ischemic-reperfusion injury was induced in mice. First, a 60-min middle cerebral artery occlusion was induced, followed by a 7-day reperfusion phase. Intracerebroventricular delivery of hAFS cells was completed, resulting in reduced neurological sequelae as well as behavioral deficits.[40] Furthermore, behavioral tests were recorded before and after the occlusion, and transplantation of hAFS cells was completed on day 35. The data suggest a lessened infarct volume, reduction in neuron loss, memory degradation, learning deficiency, and greater cell proliferation.[21] Along with promising neuroregenerative function, another study with mice showed that stem cells have neuroprotective abilities with the release of trophic factors.[41] Vascular endothelial growth factor (VEGF) was monitored and demonstrated that when overexpressed there were fewer neurological deficits and smaller infarct volumes than in mice; where VEGF was not overexpressed.[41] It is thought that this result is due to VEGF inhibition of pro-apoptotic genes such as p53.[41] Another study indicated that transplantation of ES cells into rats often formed teratomas, whereas hAFS cells did not.[42] This could be due to differences in the activity of p53 in the two cell types.

p53 is an important tumor suppressor gene in multicellular eukaryotes while also possessing apoptotic function. This gene could be a major factor in which stem cells could potentially be a therapeutic agent in stroke recovery and protection. Utilizing knowledge of p53 in the brain suggests other treatment possibilities. Research has shown that following an ischemic event, p53 mRNA and protein are upregulated, leading to an increase in p53-dependent apoptosis in the penumbra.[43] Utilizing this knowledge, treatment options arise such as a study done on methylene blue (MB) for neuroprotective function. This study found that MB modulated the p53-Bax-Bcl2-caspase3 cascade inhibiting apoptotic signaling pathways. It was also found that MB modulated the p53-5' adenosine monophosphate -activated protein kinase-Tuberous Sclerosis Complex 2- mammalian target of rapamycin cascade, enhancing autophagic signaling pathways.[44] The manipulation of p53-induced pathways with treatment shows positive results, and the studies should be continued to find new ways to manipulate p53 pathways, producing better stroke outcomes. Stem cells also need to present neural markers,[45] and p53 may provide a way to regulate these. In particular, nestin, implicated in radial growth of axons, is demonstrated to be regulated by p53. In an experiment where p53 transcription was suppressed, nestin abundance was lowered, suggesting that nestin is regulated in some way by the p53 gene.[1] Research on
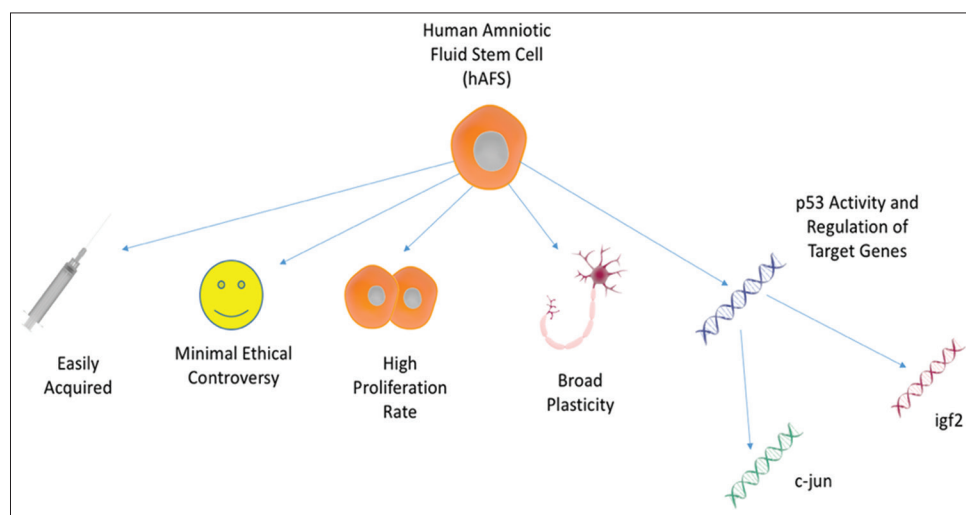

Figure 1: The use of human amniotic fluid stem cell as donor transplantable cells offers many therapeutic and logistical advantages

other areas of the body regarding ischemic-reperfusion injury has produced results that could potentially be useful in stroke research. Organ transplant is a common area with ischemic-reperfusion injury, and researchers in this field have begun looking at ischemic conditioning as a way of preconditioning the body to tolerate prolonged ischemia.[46] This runs alongside previous stroke research where stem cells are preconditioned by mild hypoxia exposure before transplanted into the brain.[47] Hypoxia causes the hypoxia-inducible factor-1 alpha to increase the expression of its target genes thought to provide neuroprotection.[48] Also proving effective as preconditioning treating is hyperbaric oxygen treatment. Introducing hyperoxia over various treatment sessions before an ischemic event can induce mild stress and prepare cells for future stressors.[49] Further research should be conducted to gain more knowledge on hypoxic and hyperoxic preconditioning to treat ischemic events.

Conclusions

Knowledge of activity and function of p53 in stem cells, the brain, and signaling pathways can lead to potential treatment options. p53 still requires much more research, especially regarding hAFS cells. However, when compared to ES cells, there are many differences that make hAFS cells a promising potential candidate for stroke therapy.

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Conflicts of interest
There are no conflicts of interest.

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