Point of View

Potential applications of intracellular antibodies (intrabodies) in stem cell therapeutics

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In recent years, there has been growing interest in the use of antibodies to target intracellular molecules [1, 2]. This has been given further impetus by the development of the single chain variable fragment (scFv) antibody format [3], which facilitated the engineering and expression of functional antibodies within the intracellular environment. The recombinant antibody expressed within the confines of the intracellular environment is commonly referred to as an intrabody. More recently, the intracellular single variable domain (IDab) format composed of only the variable region of the immunoglobulin heavy chain (V_H), has been demonstrated to make successful intrabodies that display excellent solubility, stability and expression within the cells of higher organisms [4, 5]. Indeed, this could offer a more advantageous format for intrabody construction and isolation than scFv, since there is no longer a need for phage display libraries with this approach [4].

The first reported use of an intrabody against an intracellular target was in 1988, when heavy and light-chain cDNAs of an antibody that neutralized

the yeast alcohol dehydrogenase I (ADH I) enzyme were expressed in *Saccharomyces cerevisiae* by Carlson and colleagues [6]. Since then, numerous studies have demonstrated the tremendous potential of intrabodies in various therapeutic applications [1, 2], particularly in cancer [7-9] and AIDS treatment [10-12].

At the same time, both embryonic and adult stem cells have aroused much interest over their potential applications in the newly emerging field of regenerative medicine. There are however numerous technical obstacles that have to be overcome, before widespread and routine application of stem cells in clinical therapy can materialize. Among the most formidable of these challenges include: 1) immunological barrier against allogeneic transplantation of stem cells [13], 2) teratogenic potential of embryonic stem cells [14], 3) precise control of cell lineage determination [15], and 4) limited plasticity of adult stem cells [16].

The application of intrabody technology can potentially be used to overcome these various challenges faced in utilizing stem cells for clinical ther-

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apy. Nevertheless to date, there have been no reported studies whereby these two intensive fields of research have converged. Hence, we will critically examine the prospects of applying intrabody technology to the field of stem cell therapeutics.

At this juncture, it would be appropriate to compare the use of intrabodies with alternative strategies for the targeting and inactivation of intracellular molecules; in particular RNA interference (iRNA) which acts at the level of mRNA destruction and subsequent inhibition of protein synthesis. A major technical challenge with RNA interference is the non-specific effects elicited by iRNA on the target cell. This has been demonstrated by several studies [17-20], and poses a formidable barrier to their application in clinical therapy, due to overwhelming safety concerns. For example, gene expression profiling analysis by Persengiev and colleagues [20] revealed that more than 1,000 genes involved in diverse cellular functions are non-specifically stimulated or repressed in mammalian tissue-culture cells treated with conventional 21-bp iRNAs. The use of intrabodies would circumvent this problem, since these can be designed to be highly specific to a single target protein. Another major deficiency of utilizing iRNAs is their relatively short active half-life, which would limit their effects on the cell, unless these are expressed via transfected recombinant DNA. By contrast, intrabodies being proteins possess a much longer active half-life compared to RNA, and are also much more specific to their target molecules. It would be particularly advantageous to utilize an intrabody instead of iRNA when the active half-life of the target molecule is long. In that case gene silencing through iRNA would be slow to yield any effect, whereas the effects of intrabody expression would be almost instantaneous. More importantly, if the target molecule has more than one interactive or binding domain, then gene repression through iRNA would lead to the loss of multiple functions exhibited by that particular molecule. By contrast, it is possible to design intrabodies to block certain binding interactions of a particular target molecule, while sparing others. This specificity would certainly be advantageous from the therapeutic point of view.

Apart from blocking binding interactions with the target molecule, intrabodies can also be

designed to modulate the function of its target molecule in other ways. For example, it is possible to design intrabodies to relocate its target molecule to another subcellular location *i.e.* mitochondria, nucleus or golgi apparatus, through incorporation of an appropriate localization signal [21]. Additionally, intrabodies can also be designed to promote selective degradation of its target molecule *via* the ubiquitin-proteasome pathway [22], or even death of the target cell through activation of the caspase-3 mediated apoptotic pathway [23]. All these would not be achievable through the use of RNA interference technology.

A promising area of stem cell therapeutics for the application of intrabody technology is in the downregulation of stem cell immunogenicity to facilitate allogeneic transplantation. This is particularly significant in the case of human embryonic stem cells derived from donated supernumerary embryos leftover from fertility treatment [24]; which represent a much more practical and readily-available cell-source for transplantation, as compared to obtaining isogenic embryonic stem cells from therapeutic cloning [25]. Additionally, it would also be useful to apply intrabody technology for downregulating the immunogenicity of adult stem cells, to facilitate their allogeneic transplantation. This is because the proliferative and regenerative potential of autologous adult stem cells appear to decrease with age, which limits their usefulness in older patients. Currently, there has been no reported study whereby intrabody technology has been used to downregulate the immunogenicity of either adult or embryonic stem cells. There are however a few studies with differentiated somatic cells. Mhashilkar and colleagues [26] achieved intrabody-mediated phenotypic knockout of major histocompatibility complex (MHC) class I expression in human and monkey cell lines, as well as in human primary keratinocytes; whilst Beyer and colleagues [27] achieved a similar feat with human endothelial cells for vascular transplantation.

Another major challenge in utilizing human embryonic stem cells for clinical therapy is their teratogenic potential, which poses a serious safety hazard to any potential transplant recipient. This can possibly be overcome through the application of intrabody technology. Previously, numerous studies have demonstrated the tremendous poten-

tial of intrabodies in cancer treatment [7-9]. A particularly promising approach in this case would be intracellular immunization [28] against teratoma formation, by engineering expression of intrabodies specific to target molecules implicated in teratogenicity.

Intrabodies can also be used to achieve control of cell lineage determination. Indeed, future therapeutic applications of both embryonic and adult stem cells would ultimately depend on our ability to precisely direct stem cell differentiation into well-defined lineages [15]. Conventionally, genetic modulation to direct stem cell differentiation into a required lineage is achieved through upregulated expression of lineage-specific genes, as demonstrated by the overwhelming majority of studies [15]. A much less-explored approach is to direct stem cell differentiation through repression of specific genes by either RNA interference or intrabodies. Perhaps, intrabody expression could be particularly useful for blocking stem cell differentiation into undesired lineages at the transplantation site that could impair tissue regeneration *i.e.* fibroblastic scar tissue, keloids.

Yet another potential application of intrabodies is their possible use to expand the plasticity of adult stem cells. In many cases the differentiation capacity of autologous adult stem cells appears to be strongly biased towards lineages that are characteristic of the tissue or organ from which they originate. This could in turn severely limit their application in cell transplantation therapy. Obviously it would not be possible to harvest autologous adult stem cells from diseased or damaged tissues and organs that are in dire need of therapeutic intervention. Instead it would be much more practical to extract autologous adult stem cells from other healthy tissues of the same patient and to manipulate these cells to regenerate the damaged/diseased tissue or organ. Hence it is imperative to develop strategies to expand the plasticity of autologous adult stem cells, thereby enabling them to differentiate into a greater variety of other lineages. Only then can the full potential of autologous adult stem cells in regenerative medicine be realized.

For this purpose, the application of intrabody technology appears to be particularly promising. The molecular targets of choice for intrabody-mediated inactivation are likely to be the key regulators of adult stem cell differentiation. Of particular

interest would be transcription factors that exert upstream control of somatic differentiation [29-31]. These can be thought of as "master switches" that control the expression of the entire array of proteins that are characteristic or specific to a particular lineage. Indeed, numerous transcription factors have been implicated in early lineage commitment of adult stem cells [29-31]. For example, with bone-marrow derived mesenchymal stem cells, Sox9 determines early commitment to the chondrogenic lineage [32,33] while Runx and Osterix determine early commitment to the osteogenic lineage [34, 35]. Hence if intrabody expression is used to inactivate these transcription factors, the predisposition of mesenchymal stem cells to differentiate into the chondrogenic and osteogenic lineages could possibly be reduced. This could in turn result in mesenchymal stem cells becoming more pliable to transdifferentiate into other lineages, thereby expanding their plasticity.

In conclusion, it appears that the application of intrabody technology holds much promise in the field of stem cell therapeutics. Additionally, basic scientific research in stem cell biology could also benefit from the application of intrabody technology, particularly in the area of functional genomics and proteomics [36]. Certainly, there is much to be gained from the convergence of these two intensive fields of research on stem cells and intrabodies.

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