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COMMENTARY

From Protein Synthesis to Molecular Biology: The Appealing Tale of eIF-5A

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Posttranslational hypusine modification of lysine 50 of the eukaryotic initiation factor 5A (eIF-5A) is associated with the function of this factor in cell proliferation and differentiation and is involved in tumor formation, progression and maintenance (for a review see ref. 1). In a *Molecular Therapy* Issue, Francis *et al.* report that knock down of hypusine formation in eIF-5A through RNA interference strategies is a promising approach for the treatment of B-cell neoplasia[2]. The authors had previously employed this strategy in models of multiple myeloma to abrogate hypusination of eIF-5A by codelivery of an siRNA targeted to the eIF-5A mRNA along with a plasmid encoding a mutated form of eIF-5A that can be not hypusinated.[3] The new twist in the follow-up study is that the authors have demonstrated synergistic activity of this strategy when coupled with well-established modalities of treatment of patients affected by lymphoproliferative diseases, suggesting that this approach could translate readily to a clinical setting.

Hypusine modification of eIF-5A is involved in regulation of its activity in both protein synthesis and eukaryotic cell growth. Several strategies have been used to inhibit hypusine formation. N1-guanyl-1,7-diaminoheptane (GC7), a nonspecific inhibitor of one of the two enzymes that mediate the hypusination, efficiently inhibits tumour cell proliferation. However, it gives rise to *in vivo* toxicity owing to its effect upon other enzymes.[4,5] A way to limit the toxicity of anti-cancer agents is to combine them with other nontoxic drugs that exhibit synergistic inhibition of cell proliferation. In this light, the anticancer cytokine interferon α (IFNα) showed synergistic anticancer activity with GC 7 (ref. 6) and IFNα-mediated cell growth inhibition was associated with decreased hypusine synthesis and altered eIF-5A function.[7] However, eIF-5A is expressed as two different isoforms with different functions: eIF5A1, which in its nonhypusinated form induces mytochondria-mediated apoptosis and eIF5A2, which in its nonhypusinated form loses its oncogenic potential.[8] Interestingly, GC7 also increases the response of bladder cancer cells to doxorubicin, likely due to its ability to antagonize epithelial-mesenchymal transition through inhibition of eIF-5A2 hypusination and function.[9] On these bases, the hypusination of both isoforms is essential to the oncogenic activity of eIF-5A and a benefit of the strategy used by Francis *et al.* is that it completely abrogates hypusine formation in eIF-5A without inducing nonspecific side effects.

Lymphoproliferative diseases represent an attractive target for this approach since there is an urgent need for new therapeutic strategies in chemo- or immuno-resistant patients. However, a previous report by Scuoppo *et al.* revealed a tumor suppressor function for hypusinated eIF5A in lymphoproliferative diseases.[10] These contradictory results were observed in an Eμ-Myc overexpression mouse model of lymphomagenesis that is very similar to Burkitt lymphoma, in which the lack of eIF5A hypusination promotes c-Myc-driven tumorigenesis. This is however the only report of tumor suppressor function of eIF-5A and, therefore merits additional investigation.

As noted above, the same research group demonstrated the activity of SNS01 in models of multiple myeloma.[3] This work advances the strategy reported in the previous report by demonstrating synergistic activity with bortezomib and an additive effect when combined with lenalidomide in inhibiting the growth of lymphoproliferative diseases. The use of combinatorial strategies in the treatment of cancer is an important challenge that requires determination of the optimal combination of complementary agents and their sequence of administration. The choice of optimal therapeutic strategy also depends upon the detection of predictive markers of response to therapy. In the case of eIF-5A targeted strategies, predictive biomarkers have yet to be determined and relevant animal studies are strongly warranted. Moreover, Francis *et al.* employed polyethylenimine (PEI)-based nanoparticles to deliver their nucleic acid therapy to the tumor tissues. PEI promotes self-assembly of nanoparticles so as to protect their contents from serum nucleases and is an efficient system for delivery of nucleic acids *in vivo* and in humans. However, only certain PEIs can be used for *in vivo* applications: they must exhibit an overall positive charge that allows them to bind to the negatively charged heparan sulphate proteoglycans on the cell surface. This can induce different side effects, such as liver necrosis, adhesion of aggregated platelets, shock after systemic injection at higher doses and stimulation of immune system and lung inflammation.[11] Delivery strategies can be optimized through the use of second-generation nucleic acid-nanocarriers such as stabilized nucleic acid lipid particles (SNALPs). It has been demonstrated that SNALPs are able to efficiently deliver miRNAs in the treatment of lymphoproliferative diseases and multiple myeloma without signs of toxic side effects.[12,13] SNALPs can also allow the functionalization of their surface with macrophage escaping

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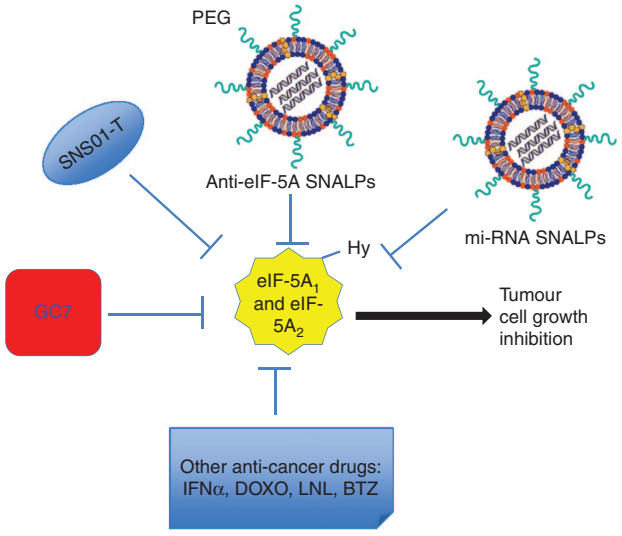

Figure 1 Historical development of molecular therapy aimed to block hypusine formation in eIF-5A. Initially GC7 was assessed to inhibit hypusine formation by enzyme inhibition (deoxyhypusine synthase inhibition) (from the right to the left). In this article, it is described a nanocarrier-based concomitant delivery of a siRNA raised against the wild type eIF-5A and of a plasmid encoding for a not hypusinable eIF-5A. Both previous strategies have been demonstrated to be potentiated by biologically rationale-based combinations with other anti-cancer agents. Possible future developments are the optimization of the nanocarriers used to deliver nucleic acids (*i.e.*, SNALPs) or the delivery of miRNAs demonstrated to be modulators of eIF-5A expression. BTZ, bortezomib; DOXO, doxorubicin; IFNα, interferon α; GC7, N1-guanyl-1,7-diaminoheptane; LNL, lenalidomide.

molecules (such as polyethylene glycol), active targeting moieties (such as transferrin) or cell penetrating peptides. It was recently reported that miRNAs can be suitable tools to inhibit cell growth through the interference with eIF-5A.[14] The advantage of miRNAs as a therapeutic derives from their ability to inhibit multiple intracellular targets and to give rise to pleiotropic interfering functions upon multiple pathways involved in tumorigenesis and cancer cell proliferation.

In conclusion, the manuscript by Francis *et al*. opens an appealing scenario in which a factor traditionally involved in protein synthesis machinery regulation becomes a molecular target for *in vivo* anti-cancer approaches. The latter are based on both the use of RNA interference techniques and nano-technology-based delivery strategies. Based on the results from Francis *et al*., exploratory clinical trials are strongly warranted and the optimization based on the search for both the optimal weapon and delivery strategy and on the finding of predictive biomarkers is still required (for a summary, see **Figure 1**).

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