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"Neural Killer" Cells: Autologous Cytotoxic Neural Stem Cells for Fighting Glioma

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Recently in *Science Translational Medicine*, Bagó et al. (2017) reported an advance in treating glioblastoma using tumor-homing cytotoxic induced neural stem cells (h-iNSC^{TE}). This approach circumvents problems associated with immune rejection and minimizes the bench-to-clinic time window critical for these patients.

Ninety years have passed since the term "glioblastoma" (GBM) was coined by Bailey and Cushing (Bailey and Cushing, 1926), and the continual search for its cure has achieved very little, despite the advances in medicine.

Treating GBM is complex due to the critical location and environment of tumors within the brain. So far, the available treatments cannot completely cure GBM due to the obscure nature of brain tumors. Recently, the conventional treatment strategies have been boosted by advances in tumor and stem cell biology. Apart from the established use of stem cells for immune reconstitution following cancer treatment, stem cells have now been widely explored as a potential drug delivery vehicle. This advent is attributed to the tumor-homing nature of neural stem cells (NSCs), particularly their ability to sense and follow chemo-attractants released by these tumors.

Bagó et al. report the use of NSCs engineered to carry cytotoxic agents for efficient treatment of GBM both in vivo and in vitro. Recently, a phase I clinical study has been completed (https://clinicaltrials.gov/; identifier: NCT01172964) in which NSCs are genetically modified to express cytosine deaminase that converts 5-fluorocytosine to cytotoxic 5-fluorouracil (Aboody et al., 2011). Two additional phase I studies have been launched: one (NCT02015819) utilizes cytotoxic 5-fluorouracil together with the drug Leucovorin, and the other (NCT02192359) implicates modified NSCs to express carboxylesterase to sensitize tumor cells in the presence of irinotecan hydrochloride.

While these clinical studies show promising results, they are all heavily based on allogeneic NSCs. Bagó et al. take these studies one step forward by using autologous patient-derived NSCs, leading to increased treatment efficacy while avoiding complications emanating from immune rejection and long-term administration of immuno-suppressants. The direct reprogramming (DR) of patient-derived fibroblast cells to NSCs (induced NSCs, or iNSCs) (Kim et al., 2011; Lujan et al., 2012) markedly reduces both the time and cost required for generating patient-specific NSCs, compared to conventional NSC derivation via transition through pluripotency, and thus decreases the risk of teratoma formation (Figure 1).

The current study reports a rapid DR strategy to convert normal human fibroblasts (NHFs) into tumor-homing iNSCs (h-iNSCs^{TE}) within 4 days through overexpression of *SOX2*. RNA sequencing (RNA-seq) analysis reveals that NSCs, and not parental NHFs, have enriched tumor-homing and cell migration pathway-associated genes (Carney and Shah, 2011). For monitoring h-iNSC^{TE} migration and therapeutic properties with respect to glioma cells, the authors generate a battery of genetically modified h-iNSCs^{TE}, glioma cells, and GBM patient-derived cells, all expressing a combination of optical and fluorescent reporters for noninvasive bioimaging and real-time motion analysis, respectively (Figure 1).

To validate the tumor-homing capacity and migration properties of h-iNSCs^{TE}, differently tagged h-iNSCs^{TE} and human GBM cells are plated next to each other separated by a gap. Motion analysis of h-iNSCs^{TE} with respect to tumor cells reveals tumor-oriented motion directionality accompanied by longer Euclidean migration distances for h-iNSCs^{TE} compared to their parent NHFs in 2D and 3D co-culture formats. Tagged h-iNSC^{TE}s are also able to penetrate into and migrate within the core of GBM-derived spheroids.

To test their therapeutic potential, the authors engineer h-iNSCs^{TE} carrying cytotoxic agents and monitor their ability

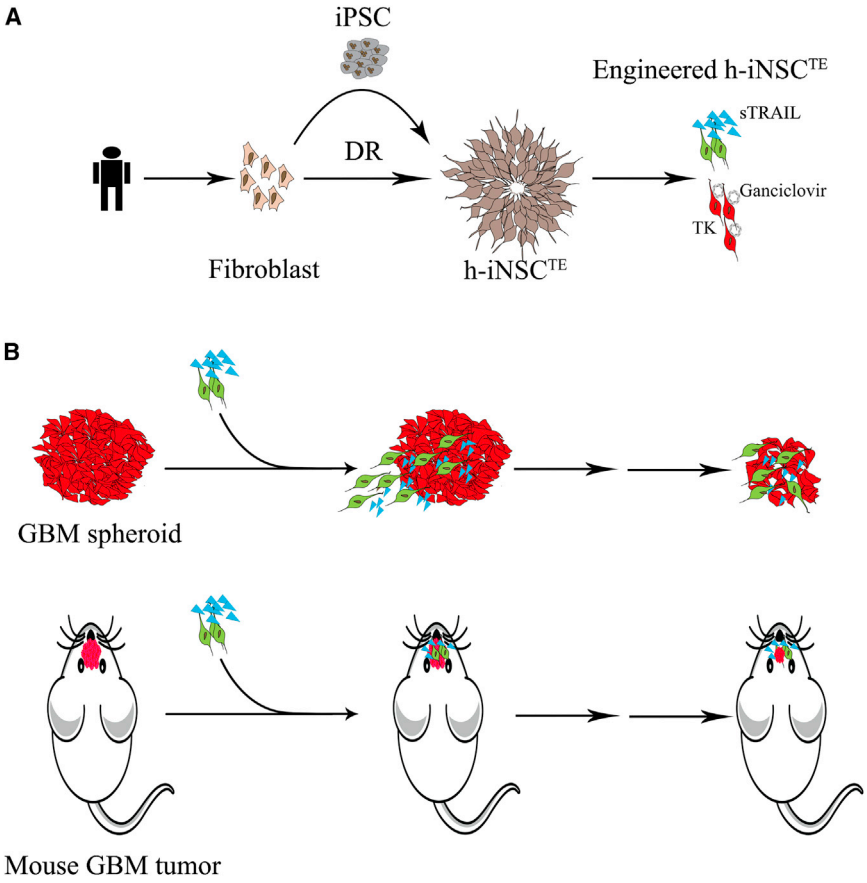

Figure 1. Rapid Generation, Engineering, and Applications of Tumor-Homing Cytotoxic h-iNSC^{TE}

(A) Time course for generation of h-iNSC^{TE} from glioma patient fibroblasts through direct reprogramming (DR) (4 days) compared with conventional iPSC differentiation schemes (~4 weeks). h-iNSC^{TE} are engineered to express different visualization tags (fluorescent and optical reporters) and cytotoxic agents (sTRAIL and TK).

(B) Monitoring migration and therapeutic applications of engineered h-iNSC^{TE}. Reduction of GBM size both in vitro and in vivo following cytotoxic h-iNSC^{TE} administration. Triangles indicate sTRAIL and circular ribbons indicate TK. (B) describes the sTRAIL paradigm but applies also to TK.

to cause tumor cell eradication in vitro and in vivo. In one paradigm, h-iNSCs^{TE} expressing the secreted pro-apoptotic molecule TRAIL (sTRAIL) are combined with tagged GBM cells to generate mixed 3D spheroids. Monitoring these spheroids in vitro over time reveals significant reduction in GBM cell viability within spheroids, in correlation with the number of applied sTRAIL-expressing h-iNSCs^{TE}. Reduction in tumor size and increased survival are also confirmed in vivo following injection of these same cell mixtures into mice brain hosts (Figure 1).

In another paradigm, h-iNSCs^{TE} are engineered to express thymidine kinase (TK) and the cells are either combined with patient-derived GBM cells to generate mixed 3D spheroids, or spheroids of both lines are first individually established and then associated in co-culture. In both cases, a marked reduction in GBM spheroid size is noted following addition of the thymidine analogue ganciclovir, which, together with TK, mediates the death of GBM cells. Furthermore, substantial reduction in tumor volume following ganciclovir administration is observed when TK-expressing h-iNSCs^{TE} are injected into brain-located tumors derived from patient GBM cells (Figure 1).

Lastly, the authors also mimic tumor recurrence using the TK paradigm. At first they generate TK-expressing h-iNSC^{TE} spheroids encapsulated in synthetic extracellular matrix (sECM) shown to permit tumor-selective migration of NSCs and increase their retention in tumor resection cavity, and they observe that h-iNSCs^{TE} reach out to adjacently placed GBM spheroids via penetrating through their encapsulating sECM. Furthermore, treating cultures with ganciclovir reduces GBM spheroid size. To mimic the recurrence pattern in mouse models, the authors employ stereotactic implantation of human invasive GBM cells into mouse brains, and then replace (by resection) the resulting tumors with TK-expressing encapsulated h-iNSCs^{TE}. Strikingly, recurrence in ganciclovir-treated mice is slower, tumor size is smaller, and mice survival is extended, demonstrating therapeutic potential for somatic cell-derived engineered NSCs.

To conclude, this study reports the rapid generation and use of autologous NSCs as tumor-homing drug carriers. It provides a clear clinical advantage over the current studies toward future development of sophisticated therapeutic setups, by utilizing tissue-matched progenitors exhibiting strong proliferative potential combined with high affinity and advantageous migration capabilities toward brain habitat—all for reaching out to tumors and combatting their aggressive nature using endogenous cell elimination paradigms while avoiding potential immune rejection and circumventing the likelihood of teratoma formation.

More broadly, this study reinvigorates intriguing future implications and potential challenges en route to the clinic. One attractive advancement would be to design distinct h-iNSC^{TE} types that match properties of various tumor types. It is known, for example, that early human embryonic stem cell (hESC)-derived NSCs exhibit increased overgrowth potential following grafting compared to later-derived NSCs that tend to migrate, integrate, and differentiate within the host brain (Elkabetz et al., 2008). Accordingly, hESC-derived neural progenitors carrying a common pediatric glioma mutation in histone H3.3 express primitive, but not late, NSC gene sets (Funato et al., 2014). Therefore, enrichment analysis of transcriptional and epigenetic maps for distinct types of NSC stages (Elkabetz et al., 2008; Edri et al., 2015; Ziller et al., 2015) within gene signatures obtained from specific tumor types should result in a sophisticated designer h-iNSC^{TE} with tumor-matched prolonged therapeutic durability. Other more generalized strategies to control the proliferative nature of h-iNSCs^{TE} include designing a neuronal precursor-like version of h-iNSCs^{TE} bearing limited proliferation capacity, or h-iNSCs^{TE}

expressing a modified TK of reduced substrate affinity. As TK expression is designed to be induced only in h-iNSCs^{TE}, but not tumor cells, it will be interesting to elucidate mechanisms by which TK-expressing h-iNSCs^{TE} mediate tumor cell eradication (possibly by the bystander effect), which should advance harnessing the full potential of NSCs for long-term, controlled eradication of tumor cells.

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lncRNA-Encoded Polypeptide SPAR(s) with mTORC1 to Regulate Skeletal Muscle Regeneration

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Although prematurely baptized as non-coding, some lncRNAs encode polypeptides with regulatory functions that are implicated in various biological processes. Matsumoto et al. (2017) recently report in *Nature* that LINC00961 generates SPAR polypeptide that acts via the lysosome to suppress amino-acid-mediated mTORC1 activity, thereby modulating skeletal muscle regenerative response following injury.

Since their discovery, long non-coding RNAs (lncRNAs) have reshaped our thinking on the design and regulatory landscape of genomes in metazoans. lncRNAs bear some of the hallmarks of mRNAs, as they are transcribed by RNA polymerase II, spliced, capped, and polyadenylated, yet they have been initially surmised to have little to no open reading frame (ORF) information. lncRNAs are generally less widely expressed than mRNAs, thus raising the notion that they actively regulate specific biological processes (Ulitsky and Bartel, 2013). For example, *Xist* lncRNA is transcribed from the X chromosome, and it acts in cis to promote its inactivation (Penny et al., 1996; Ulitsky and Bartel, 2013). lncRNAs can also directly modulate the activity of enhancer elements and maintain a permissive chromatin state (Anderson et al., 2016). However, in defiance of their name, some lncRNAs are reported to harbor relatively small regulatory polypeptides whose functions have been challenging to decipher.

To address this issue, Matsumoto and colleagues (Matsumoto et al., 2017) use a proteomics strategy to identify novel lncRNA-encoded polypeptides. This screen unveils a 90-amino-acid (aa) polypeptide (ORF1) encoded by lncRNA LINC00961 that is expressed at high levels in lung, heart, and skeletal muscle in both human cells and mice (Figure 1A). ORF1 localizes to late endosomes and lysosomes, and mass spectrometry studies show that ORF1 interacts with subunits of the v-ATPase proton pump and the lysosome, but it does not play a role in its localization or its activity. Furthermore, in response to aa stimulation, the v-ATPase complex has been shown to interact at the lysosome with the Ragulator complex to regulate activation of mTORC1 (mechanistic target of rapamycin 1) kinase. mTORC1 regulates anabolic and catabolic processes by acting as a sensor of nutrient, redox, and energy levels. It localizes to the surface of lysosomes to phosphorylate ribosomal proteins (S6 kinase and S6) and an inhibitor of translation, 4E-BP1, thereby promoting translation initiation and protein synthesis (Laplante and Sabatini, 2013). Following stimulation by specific amino acids, v-ATPase/Ragulator complexes and GTPases promote the translocation of mTORC1 to lysosomes (Figure 1B). To examine mTORC1 activation, the authors first starve then stimulate human HEK293T cells stably