Reprogrammed immune cells might give doctors an edge in rallying the body's defenses against tumor growth

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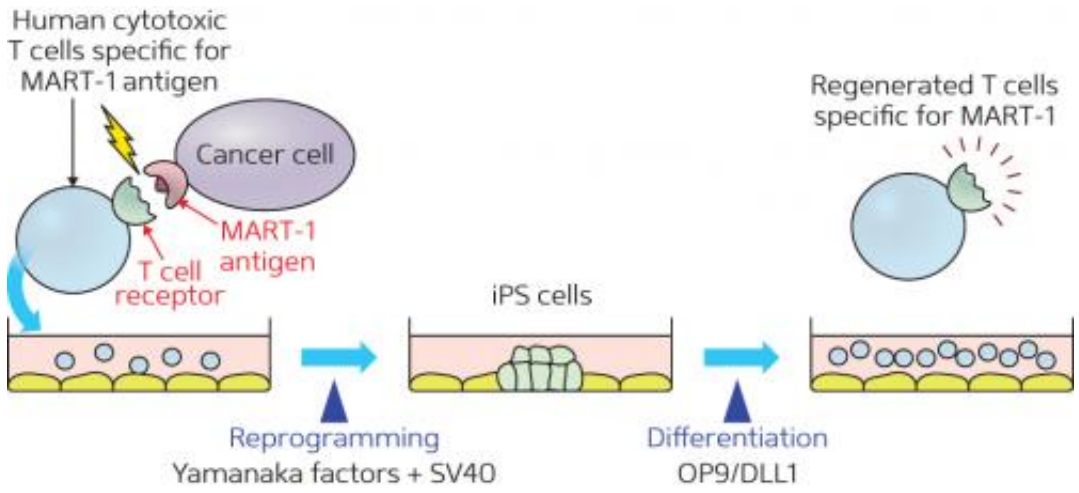

Figure 1: Scientists reprogrammed purified CTLs into embryonic stem cell-like iPSCs by forcing them to express genes known as the 'Yamanaka factors'. After allowing these reprogrammed cells to proliferate, they then cultivated these iPSCs alongside specialized cells (OP9/DLL1) that promote their maturation into transplantation-ready, MART-1-specific CTLs. Credit: 2013 Elsevier

Genetic abnormalities accrued by tumor cells lead to inappropriate production of proteins at the wrong time or place, or even the synthesis of unusual hybrid proteins not found in normal cells. Such abnormalities can serve as 'red flags' that alert the immune system that something has gone awry, triggering proliferation of cytotoxic T lymphocytes (CTLs)

that can recognize and destroy defective cells based on these protein signatures.

Unfortunately, cancers ultimately deploy defensive strategies that render the body's natural immune response incapable of stopping cancerous growth, and scientists have encountered only limited success with vaccines and other strategies that help 'super-charge' the anti-tumor immune reaction. Now, new stem cell research by Hiroshi Kawamoto and colleagues at the RIKEN Research Center for Allergy and Immunology promises to greatly bolster the effectiveness of such approaches1.

Although the concept of vaccines based on tumor-specific antigens is sound, the generation of a CTL response sufficient to overwhelm a cancer's defenses has proved elusive. "The number of potent anti-cancer cells is small and even if they are efficiently activated, their life span is short," explains Kawamoto. "With these methods, effects can be seen in 10–30% of cases, but this does not necessarily mean a cure—just slightly prolonged survival." To address this challenge, Kawamoto and his colleagues pursued a strategy for producing far greater numbers of patient-specific antitumor CTLs.

Made to order

Stem cells represent an ideal resource for the production of patient-specific antitumor CTLs, but the production process poses special challenges. To deal with the vast array of potential threats that might endanger our health, the immune system uses a complicated gene-shuffling process called recombination to generate armies of cells that each express receptors capable of recognizing a different target. This means that CTLs produced from either embryonic or adult stem cells would hardly retain the target-recognition capabilities needed to kill a tumor.

Kawamoto's team therefore pursued an alternative approach involving the use of a genetic reprogramming technique to transform mature human CTLs into induced pluripotent stem cells (iPSCs), which closely resemble embryonic stem cells in terms of their developmental flexibility. From this undifferentiated state, iPSCs can be cultivated under conditions that favor their development into mature CTLs (Fig. 1). Importantly, as the genes of the parental cells are pre-shuffled, the end result should be a large quantity of targeted CTLs. "All of these lymphocytes will come to express the same antigen receptor as the original T cells," says Kawamoto. "The technique allows us to regenerate antigen-specific T cells with very high efficiency."

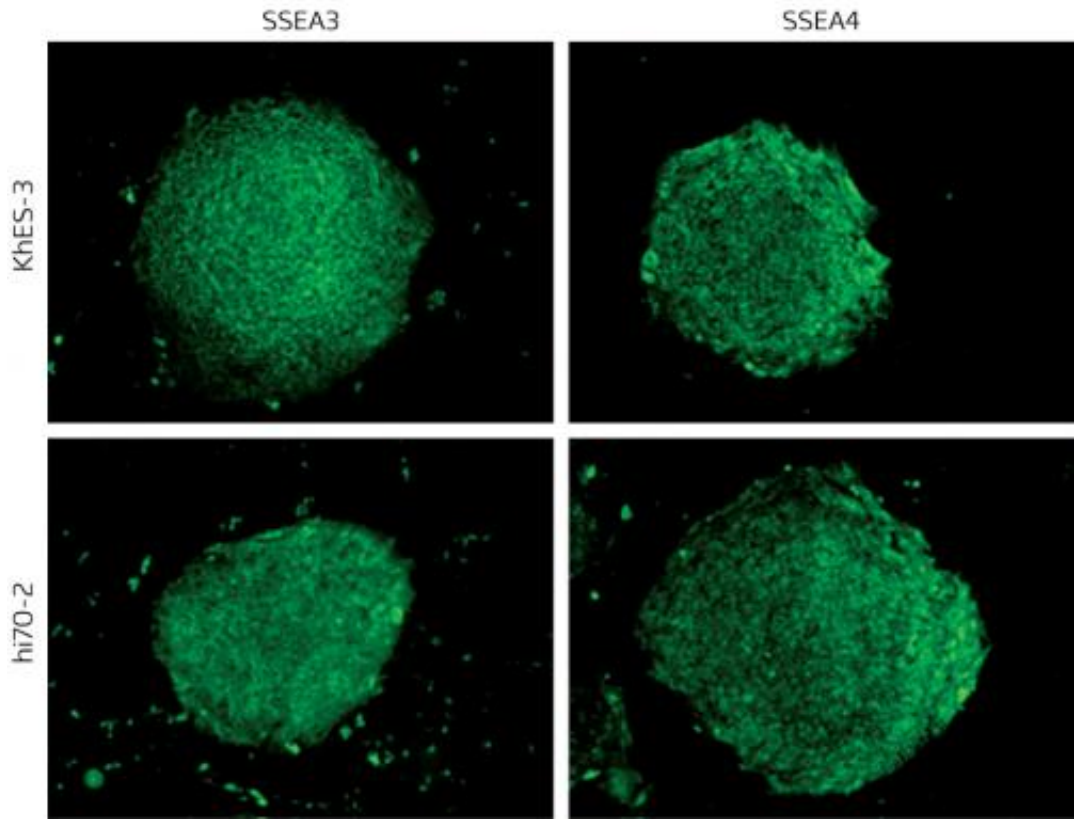

Figure 2: Human embryonic stem cells (KhES-3) express a number of specific genes that enable them to maintain their developmental flexibility, such as SSEA3 and SSEA4. CTL-derived iPSCs (hi70-2) express these genes at levels that are essentially indistinguishable from embryonic stem cells, demonstrating

that they also retain the flexibility for subsequent production of mature immune cells. Credit: 2013 Elsevier

After proving the soundness of this approach using an isolated pool of mixed human CTLs, the researchers applied their method to a specific line of CTLs that selectively recognize MART-1, a protein commonly overexpressed by the skin cancer melanoma. After reprogramming, they obtained two iPSC lines that contained the same recombined MART-1-specific receptor gene found in the parental CTL cell line (Fig. 2). Using one of these as starting material, the researchers were able to produce a 95%-pure pool of new anti-MART-1 CTLs.

Initial experiments using the purified MART-1 confirmed that these iPSC-derived CTLs were producing the specialized receptor required to bind this melanoma antigen. Immune cell activation typically entails the production and release of specialized signaling molecules called cytokines, and Kawamoto and his colleagues confirmed that their CTLs produced appropriate cytokines when cultured alongside human cells that express MART-1 on their surface. This result is encouraging, as the experiment roughly replicates the context in which CTLs might encounter this antigen in a melanoma patient.

Testing the troops

The question of whether this apparent tumor-specific immune response will translate into improved patient outcomes remains to be determined, and this will be a top focus of Kawamoto's team moving forward. "We wish to test whether the regenerated T cells can kill tumor cells in in vivo situations using mouse models," he says. "We are also planning to try the same experiments using different tumor antigens to confirm that this method can be generally used."

If the experiments prove successful, this technique could benefit large numbers of cancer patients. In addition to melanoma, scientists have categorized a broad range of potentially useful target antigens from a host of different tumors, including common killers such as breast, lung and colorectal cancer. Experimental vaccines are already in the developmental pipeline for several of these diseases, and their performance could be dramatically enhanced by coupling with techniques that can help doctors to quickly raise vast quantities of patient-specific CTLs. These benefits need not be limited to cancer patients, and Kawamoto notes that their iPSC-based approach could also help patients mount more effective defenses against particularly intractable infections, such as human immunodeficiency virus (HIV).

Several other technical challenges also remain to be addressed. The initial preparation of iPSCs remains a time-consuming and inefficient process, and the isolation of high-quality, tumor-specific 'source' CTLs from patients will likewise require significant effort. However, Kawamoto believes that this could be a valuable clinical proving ground for these cells, which have garnered considerable attention in the research community—including last year's Nobel Prize—but have not yet begun to achieve their perceived potential in the world of human regenerative medicine. "iPSC technology is being applied primarily to the compensation of lost or damaged tissues, but only a relatively limited number of patients require tissue regeneration," says Kawamoto. "If this technology can be applied to cancer therapy, an extremely large number of patients would benefit."

More information: Vizcardo, R., Masuda, K., Yamada, D., Ikawa, T., Shimizu, K., Fujii, S., Koseki, H. & Kawamoto, H. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8+ T cells. *Cell Stem Cell* 12, 31–36 (2013). dx.doi.org/10.1016/j.stem.2012.12.006

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