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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Unraveling Tumorigenicity in iPSC-Derived Immune Cells: The Impact of Chromosomal Abnormalities and Proliferative Intermediates

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Response to tumor cells by the immune system can determine the course of a cancer. This guiding principle and the dynamic, adaptable nature of our immune system underlies the drive to generate immune cells for cancer immunotherapy. Induced pluripotent stem cells (iPSCs) provide an unlimited resource for the generation of immune cells such as T cells and dendritic cells (DCs) for cancer immunotherapy. However, the use of iPSC-derived immune cells in clinical applications has been limited due to concerns of safety. In vitro passage of iPSCs can introduce selective pressures that promote the accumulation of mutations that confer a growth advantage. Over several passages, this can lead to the acquisition of chromosomal abnormalities. Moreover, differentiation protocols of varying efficiency permit the presence of immature cells, contaminating the differentiated cell population. However, the impact of chromosomal abnormalities and intermediate products with proliferation potential on tumorigenicity of iPSC-derived immune cells remains unclear.

Here, we investigated the tumorigenic potential of iPSC-derived T cells and DCs in immunocompetent and immunodeficient mice. Pmel-1 T-cell receptor (TCR) transgenic mouse T cells were reprogrammed using Sendai virus vectors carrying Oct3/4, Sox2, Klf4 and cMyc. While our iPSCs were phenotypically normal, even at early passage numbers, various chromosomal abnormalities were found in the resulting cell population. These abnormalities included trisomy 8 and 11 and loss of X chromosome with high frequency. Furthermore, within a population of iPSCs determined to be karyotypically normal by G-banding, unbalanced translocations were found by spectral karyotyping. These findings are consistent with those reported in the literature and highlight the prevalence of chromosomal abnormalities in iPSC populations. As iPSCs can harbor chromosomal abnormalities that are undetectable by standard methods, we assessed the tumorigenic potential of iPSC-derived cells, similar to what may be used in clinical applications.

Mouse iPSCs were differentiated into CD8$\alpha\beta$ T cells by co-culture with murine OP9 bone marrow stromal cells expressing the Notch ligand Delta-like 1 (OP9-DL1) and were enriched by CD8 positive selection. Activated iPSC-derived T cells were adoptively transferred to immunocompetent C57BL/6 and immunodeficient NOD/SCID, IL-2 receptor γ chain knockout mice. Immunodeficient but not immunocompetent mice developed hepatic tumors. Reverse transcription (RT)-PCR identified Pme1-l TCR gene in tumor cells, confirming tumor origin. Moreover, histology of liver tumors is suggestive of lymphoma or lymphosarcoma. Within the population of CD8+ iPSC-derived cells, a small subpopulation co-expressing CD8 and SSEA-1 was present. Sorting the cells by SSEA-1 positivity eliminated tumor formation in vivo.

Mouse iPSCs were also differentiated into DCs by co-culture with OP9-DL1 cells and granulocyte macrophage colony-stimulating factor. Despite similar use of iPSCs with chromosomal abnormalities and prolonged passaging in vitro, SSEA-1+ cells were not identified when they were differentiated to DCs, and iPSC-derived DCs did not form tumors in immunodeficient mice.

Taken together, this study revealed the potential risk of tumorigenicity of iPSC-derived immune cells in our preclinical model. The presence of chromosomal abnormalities did not definitively determine risk of tumorigenesis upon transplantation. The relationship between SSEA-1 positivity and tumor formation suggests contaminating cells may carry tumorigenic charac-

teristics. Further work is required to develop differentiation methods that generate pure populations, free of tumorigenic intermediates, to potentiate the clinical use of iPSC-derived immunotherapy.

Disclosures No relevant conflicts of interest to declare.

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