Final Report:

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The project title: **Human disease model of PSC for discovery of effective PSC therapy**

1. Research summary including milestones achieved and any challenges identified:
Scientific accomplishments of the award period 2020-2022.

With the support of the two years of PSC partners research grant award, we have been able to establish a method of generating iPSC (induced pluripotent stem cell) lines from patients' peripheral blood samples. All iPSC lines generated from the integration free method expressed a variety of pluripotency associated markers and characteristics (the goals of specific aim 1). In the year 2, we have expanded the established iPSC lines (Figure 1) and investigated their hepatobiliary differentiation potential compared with non-PSC lines (the goals of specific aim 2).These stem cell lines were able to be differentiated into hepatobiliary lineage cells however showed a higher level of fibrosis markers compared to the control lines in our preliminary studies (Figure 2).

We have accomplished all the goals of our specific aims of this original application, which are focused on establishing, characterizing and differentiating human iPSC lines from patient derived tissues. Please see below data obtained from the newly established human iPSCs generated from PSC patients (Figure 1) and their differentiation characteristics that are different from normal control lines (Figure 2). With further studies using additional patient stem cell lines in the future, we will be able to establish fully characterized disease model of PSC. Since there is currently no human model and drug treatment for PSC patients, the resulting cellular models based on patient iPSCs will be highly useful for disease mechanism research and performing drug discovery for PSC therapy.

2. List of publications and abstracts presented:

1. Wang L, Ye Z, **Jang YY**. Convergence of human pluripotent stem cell, organoid, and genome editing technologies. *Exp Biol Med* (Maywood). 2021; 246(7):861-875. PMID: 33467883
2. Wang Y, **Jang YY.** From Cells to Organs: the Present and Future of Regenerative Medicine. *Adv Exp Med Biol*. 2021 Jul 31. doi: 10.1007/5584_2021_657. Online ahead of print. PMID: 34327664
3. Wang Y, **Jang YY**. Human iPSCs for Modeling of Hepatobiliary Development and Drug Discovery. Adv. *Stem Cell Biol*. 2022; 16: 95-109
4. Tian L, Wang Y, **Jang YY.** Wnt signaling in biliary development, proliferation, and fibrosis. *Exp Biol Med*. 2022 Feb; 247(4):360-367. PMCID: PMC8899336
5. Tian L, Ye Z, **Jang YY**. Generation and differentiation of disease-specific human induced pluripotent stem cell line from Primary Sclerosing Cholangitis patients. *In Preparation.*

3. Key Data (Generation and differentiation of patient iPSC lines from PSC patients).

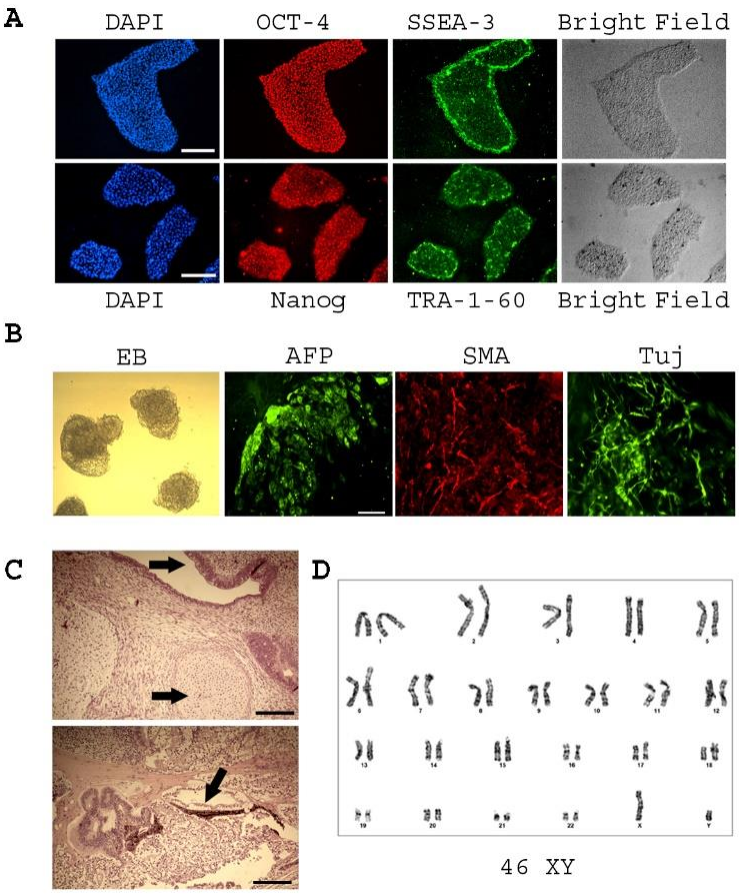

Figure 1. Characterization of PSC patient-specific iPSC line.
(A) Pluripotent marker expression of PSC patient-iPSC line. Representative immunofluorescence analysis of one patient iPSC line growing on Matrigel. Clear expression of embryonic stem cell surface antigens SSEA-3 and TRA-1-60 and nuclear transcription factors OCT4 and NANOG can be observed (×200; scale bar = 50 μm). (B, C) These iPSCs can differentiate into all three primary germ layers in vitro and in vivo. (B) Embryoid bodies (EBs) derived from the PSC patient iPSC. In vitro differentiation of these iPSCs into all three primary germ cell layers. After the generation of EBs, the PSC-iPSC lines spontaneously differentiated into endoderm (AFP-positive, green), mesoderm (SMA-positive, red), and ectoderm (TuJ1-positive neuronal cells, green). The blue nuclear staining was 4′,6-diamidino-2-phenylindole (×100; scale bar = 100 μm). (C) Spontaneous differentiation into all three germ layers was evident in teratomas: endoderm (glandular epithelium), mesoderm (immature cartilage), and ectoderm (pigmented epithelium) derived from the PSC-patient derived iPSCs (×200; scale bar = 50 μm). (D) Twenty metaphases were analyzed and a normal male karyotype was observed. No consistent chromosome abnormalities were detected in this specimen.

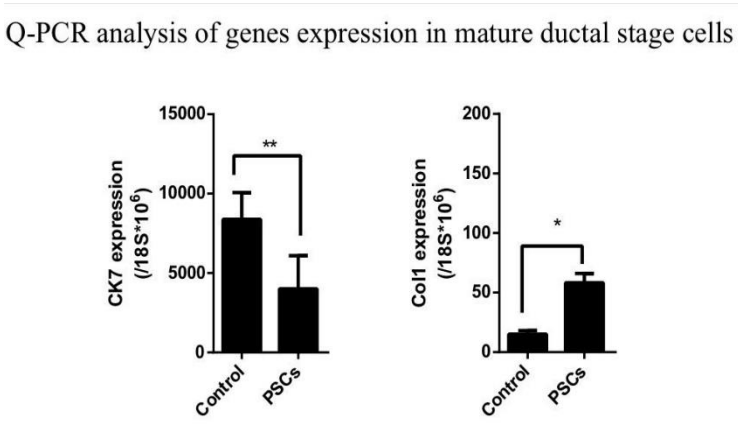

Figure 2. Increased fibrosis marker gene expression in biliary cells differentiated from PSC patient-specific iPSC lines.
Quantitative PCR analysis of a cholangiocyte marker CK7 (left panel) and a fibrosis marker Collagen type 1 (right panel) in the mature stage biliary ductal cells from PSC patient derived iPSCs. Although preliminary, patient lines expressed significantly higher level of collagen and lower CK7 suggesting the patient cells recapitulating a key disease phenotype, fibrosis, in a dish.

4. Lay summary and impact: brief paragraph, for the PSC Partners community and for the lay public, summarizing the progress of your research project.

This project is designed to address an unmet medical need for the treatment of primary sclerosing cholangitis (PSC). Many patients suffer from fibrosis of the bile ducts and liver. Unfortunately, there is no effective treatment for PSC. This is partly due to the lack of a well-defined human cellular model of the disease that would allow mechanistic studies of pathogenesis or development of drugs. Available cell lines such as cholangiocarcinoma lines, are not relevant to PSC and do not recapitulate fibrosis. This is why we chose to develop the disease-relevant human model of PSC, based on our expertise in patient induced pluripotent stem cells. The PSC partners Research Grant Award has provided timely important support for pursuing this new research direction of PSC study in my laboratory. With the support of the two year pilot fund, we have been able to initiate the project of establishing an essential method of generating patient-specific pluripotent stem cells from multiple PSC patients' tissues. These PSC patient derived stem cells expressed various markers and specific features of pluripotent potent stem cells, and this further allowed us perform differentiation studies which is a basis of disease modeling in the second year. This progress is critical for us to establish the necessary foundation towards developing preclinical human PSC model. With further research with additional patient lines and extensive differentiation and characterization studies, these patient-derived stem cells will be utilized for modeling the PSC disease features in a dish. The novel PSC disease model will help understand currently unclear disease mechanisms of this disease and accelerate developing new drug therapy for PSC patients.