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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte SHINYA YAMANAKA and MICHIYO KOYANAGI

Appeal 2014-006588
Application 12/379,564[1]
Technology Center 1600

Before DONALD E. ADAMS, JOHN G. NEW, and
KRISTI L. R. SAWERT, *Administrative Patent Judges.*

NEW, *Administrative Patent Judge*.

DECISION ON APPEAL

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[1]Appellants state the real party-in-interest is Kyoto University. App. Br. 1.

SUMMARY

Appellants file this appeal under 35 U.S.C. § 134(a) from the Examiner's Final Rejection of claims 1–5, 7, and 9–11, which stand rejected as unpatentable under 35 U.S.C. § 112(a) for lack of enablement.

We have jurisdiction under 35 U.S.C. § 6(b).

We REVERSE.

NATURE OF THE CLAIMED INVENTION

Appellants' invention is directed to a method of preparing induced pluripotent stem cells, comprising a nuclear reprogramming step with a nuclear reprogramming factor in the presence of micro RNA ("miRNA"), wherein said miRNA has a property of providing a higher nuclear reprogramming efficiency in the presence of said miRNA than in the absence thereof. Abstract.

REPRESENTATIVE CLAIM

Claim 1 is representative of the claims on appeal and recites:

> 1. A method of preparing an induced pluripotent stem cell from a mammalian somatic cell, comprising nuclear reprogramming the mammalian somatic cell under culture conditions which allow for mammalian somatic cell reprogramming to pluripotency with at least one miRNA and an expression vector encoding at least one nuclear reprogramming factor,
>
> wherein the at least one miRNA increases efficiency of the nuclear reprogramming of the mammalian somatic cell compared to nuclear reprogramming of the mammalian somatic cell with the expression vector in the absence of the at least one miRNA,

> wherein the at least one miRNA is one or two or more miRNAs contained in one or two or more RNAs selected from the group consisting of: hsa-miR-372; hsa-miR-373 or hsamiR-373/373*; hsa-miR-371-373 cluster; hsa-miR-302b or hsa-miR-302b/302b*; hsa-miR-302-367 cluster; hsa-miR-520c or hsa-miR-520c-5p/520c-3p; mmu-miR-291a or mmu-miR-291a-5p/291a-3p; mmu-miR-294 or mmu-miR-294/294*; and mmu-miR-295 or mmu-miR-295/295*, and
>
> wherein the at least one nuclear reprogramming factor comprises at least (i) an Oct3/4 gene, (ii) an Oct3/4 gene and a Klf4 gene, (iii) an Oct3/4 gene and a Nanog gene, or (iv) an Oct3/4 gene, Klf4 gene, and a Myc family gene, but it does not comprise a Sox family gene.

App. Br. 16.

ISSUES AND ANALYSES

We neither agree with, nor adopt, the Examiner's findings and conclusion that the appealed claims are not enabled as required by 35 U.S.C. § 112(a). We address the arguments raised by Appellants on appeal below.

Issue

Appellants argue the Examiner erred in concluding that the claims were not enabled based in the Examiner's finding that, at the time of invention, a retroviral vector was the only means by which to deliver genes of nuclear reprogramming factors and induce an iPSC.

Analysis

The Examiner concluded that "[t]he claims are not enabled as presently written because the art at the time of filing regarded retroviral

vectors as the only means through which to deliver genes of nuclear reprogramming factors sufficiently to produce an iPSC ["induced pluripotent stem cell"]. App. Br. 6 (quoting Final App. 4). Appellants dispute this conclusion, arguing that the Examiner misconstrued the state of the art at the time Appellants' application was filed. *Id.*

Appellants point to M. Stadtfeld et al., *Induced Pluripotent Stem Cells Generated without Viral Integration*, 322 SCIENCE 945–49 (November 7, 2008) ("Stadtfeld"), which, Appellants argue, discloses fibroblasts, and adult mouse hepatocytes with adenoviral vectors comprising genes encoding Oct4, Sox2, KIf4 and c-myc. App. Br. 7 (citing Final Act. 5). Appellants also point to K. Okita et al., *Generation of Mouse Induced Pluripotent Stem Cells without Viral Vectors*, 322 SCIENCE 949–53 (November 7, 2008) ("Okita") which, Appellants contend, discloses nuclear programming with non-retroviral expression vectors. *Id.* According to Appellants, Okita discloses the use of expression plasmids to induce iPSCs from mouse embryonic fibroblasts in the presence of miRNAs. *Id.* Appellants note that their Specification explicitly discloses both Stadtfeld's and Okita's techniques of using adenoviral and plasmid vectors, respectively, to deliver nuclear reprogramming factors (or genes). *Id.* (citing, e.g., Spec. ¶ 108).

Appellants argue that it was well known in the art at the time of Appellants' filing that a gene introduced via a retrovirus is transcribed into a protein that subsequently performs its function. App. Br. 8. Appellants assert that multiple gene transfer methods that achieve this same result were also well known at the time of filing. *Id.* Therefore, Appellants contend, because retroviral delivery was one of multiple known gene transfer methods, it would not have required undue experimentation for one of

ordinary skill in the art to practice gene transfer methods other than a retroviral delivery method in practicing the present invention. *Id.*

Appellants dispute the Examiner's finding that the claims are not enabled because the claims are not limited to the specific cell types demonstrated in Stadtfeld and that "for plasmid delivery of nuclear programming factors, the specification does not disclose an enabling embodiment and none has been cited by appellant." App. Br. 8 (quoting Final Act. 5). Appellants contend that: (1) transfection methods reported in Stadtfeld and Okita were known in the art at the time filing; and (2) "[a] patent specification need not teach, and preferably omits, what is well known in the art." *Id.* (quoting MPEP § 2164.01).

According to Appellants, the state of the art at the time of filing included knowledge of non-retroviral vectors for generation of iPS cells regardless of whether embodiments of the present invention with those methods are included in the Specification. *Id.* Appellants argue that, as Stadtfeld and Okita demonstrate, gene transfer technology was mature at the time of filing. *Id.* at 8–9. Moreover, argue Appellants, the Specification explicitly directs the reader to multiple references that disclose methods for delivering reprogramming genes to a target cell. *Id.* at 10 (citing, e.g., Spec. ¶¶ 108, 134).

Appellants also dispute the Examiner's finding that, because only methods employing a retrovirus are disclosed in the working examples of Appellants' Specification, it would require undue experimentation by a person of ordinary skill to practice other gene transfer methods. App. Br. 10. In response, Appellants point to MPEP § 2164.01(b), which states that: "[a]s long as the specification discloses at least one method for making and

using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied." *Id.* Furthermore, Appellants assert, the enablement requirement of § 112 does not necessarily require disclosure of a working example. *Id.*

The Examiner responds that Stadtfeld and Okita, both published on November 7, 2008 cannot be considered "well-known in the art at the time of filing," when the priority date of the instant application is November 25, 2008. Ans. 11. The Examiner finds three weeks is insufficient time to establish knowledge as "well-known." *Id.* Rather, the Examiner finds, only knowledge related to the production of retroviral vectors can be considered well-known in the art. *Id.* at 11–12.

Furthermore, the Examiner finds, in summarizing the state of the art at the time of filing, an article published by one of the inventors of the instant application teaches the use of a retroviral transfection system comprising nucleic acid sequences encoding reprogramming factors allegedly indispensable for iPS cell induction. Ans. 12 (citing S. Yamanaka, *Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors* 41, Suppl. 1 CELL PROLIFERATION, 51–56 (2008) at 55) ("Yamanaka"). The Examiner finds that, although Yamanaka states other factors may induce iPSCs without a need for retroviruses, these had yet to be identified. *Id.* (citing Yamanaka 55). Therefore, the Examiner finds, the art at the time of filing regarded retroviral vectors as necessary to reprogramming methods. *Id.*

The Examiner admits that, at the time of filing, there was little unpredictability in the production of expression vectors, the transduction of cells in culture and the expression of functional protein. Ans. 13. However,

the Examiner finds, the claims are not directed to the production of a functional protein in transduced cells, rather the reprogramming factors are required to reactivate genes expressed during embryonic development that had been inactivated during cell differentiation. *Id.* The Examiner finds the unpredictable nature of the claimed invention is in the employment of other, non-retroviral, vectors exhibiting sufficient expression to activate the appropriate pluripotency genes such that they maintain the induced cell in a pluripotent state. *Id.* The Examiner finds non-retroviral vectors, as exemplified by adenovirus, cannot predictably express reprogramming factors sufficiently to induce reprogramming of a somatic cell and, therefore, the Examiner finds more than mere expression of nuclear reprogramming factors is required to induce somatic cells to revert to a pluripotent state. *Id.* at 13–14.

The Examiner further finds that, given the low efficiency of induction disclosed in Appellants' Specification, even if co-induced with miRNAs, Appellants' claimed method requires more than just expression of the exogenous nuclear reprogramming factor genes. Ans. 14. The Examiner finds that if mere expression of the exogenous factor genes met the requirement for reprogramming, the efficiency would be considerably higher. *Id.* (citing Spec. Table 3; ¶ 73). The Examiner finds that these results disclosed in the Specification indicate that retroviral vectors do not guarantee the production of iPSCs. *Id.* Consequently, the Examiner finds, when the alleged retroviral failure is taken with Stadtfeld's failure to obtain iPSCs with adenovirus delivery of reprogramming factor genes, Appellants' invention, as presently claimed, lacks predictability without undue experimentation. *Id.* Consequently, the Examiner concludes, the data

disclosed in Appellants' Specification, and in the art at time of filing, clearly shows that delivery of reprogramming factor genes by non-retroviral vectors lacked enablement at the time of filing. *Id.*

We are persuaded by Appellants' arguments. Appellants' Specification explicitly discloses, in addition to the use of a retroviral transfection vector in the examples, other prior art references teaching different vectors. Specifically, the Specification discloses:

> Methods for using a lentivirus as a vector, as well as another combination of nuclear reprogramming factor genes, are disclosed in Yu et al., *Science* **318**: 1917–1920, 2007; and *Cell Stem Cell* **3**: 568–574, 2008, which are herein incorporated by reference in their entirety. Methods for using adenovirus as a vector are disclosed in Stadtfeld et al., *Science* **322**:945-949, 2008, which is herein incorporated by reference in its entirety. Methods for using a plasmid as a non-viral vector are disclosed in U.S. Provisional Application No. 61/071,508; U.S. Provisional Application No. 61/136,246; U.S. Provisional Application No. 61/136,615; and U.S. Provisional Application No. 61/193,363entitled "Method for Nuclear Reprogramming" filed November 21, 2008; and Okita et al., *Science* **322**: 949-953, 2008, which are herein incorporated by reference in their entireties. One of ordinary skill in the art could choose and use an appropriate method from among the above known methods, or from any of the other known methods or vectors available in the prior art.

Spec. ¶ 108. The Specification further discloses:

> For introduction of a vector or miRNA, known techniques, such as microinjection, liposome, lipofection, electroporation, cal[c]ium phosphate, and viral infection, may be used. The viral infection using retrovirus or [l]entivirus vector is described in prior art (e.g., *Cell*, **126**: 663–676, 2006; *Cell*, **131**:861–872, 2007; or *Science*, **318**: 1917–1920, 2007). The use of adenovi[r]us vector is described in *Science*, **322**: 945–949

> (2008), wherein specific means for use of a plasmid is taught too (*Science*, **322**:949-953 (2008)).

Spec. ¶ 134. We agree with Appellants that the Specification thus explicitly points to, and specifically incorporates by reference, prior art that teaches alternative transfection vectors to a retrovirus.

Okita teaches:

> [T]he generation of mouse iPS cells without viral vectors. Repeated transfection of two expression plasmids, one containing the complementary DNAs (cDNAs) of Oct3/4, Sox2, and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without evidence of plasmid integration….

Okita 949. Specifically, Okita teaches:

> [T]he efficiency of iPS cell induction with the plasmid transfection protocol is substantially lower than that with the retroviral method. Nevertheless, we obtained iPS cell clones without evidence of plasmid integration in 6 out of 10 experiments (figs. S2 to S5), demonstrating reproducibility of the protocol.

Okita 952.

Stadtfeld teaches that, although induction of iPS cells using an adenovirus transfection vector is less efficacious than via a retroviral vector, the protocol is generally successful and reproducible (and thus enabled) and, moreover, teaches that use of an adenovirus vector mitigates the risk of retroviral tumorigenicity. *See* Stadtfeld 945. ("[A] major limitation of this technology is the use of [retro]viruses that integrate into the genome and are associated with the risk of tumor formation due to the spontaneous reactivation of the viral transgenes").

Similarly, Okita teaches: "Repeated transfection of two expression plasmids, one containing the complementary DNAs (cDNAs) of Oct3/4, Sox2, and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without evidence of plasmid integration." Okita Abstr. Okita similarly reports a 60% success rate in generating iPS cell clones using a plasmid as a transfection vector and, as in Stadtfeld, teaches that reduced tumorigenicity is an advantage of this method when compared to use of retroviral vectors. Okita 952; Abstr. Okita also teaches that using a lentivirus as a transfection vector was also known in the art at the time of filing. *See* Okita Abstr.

Yamanaka, cited by the Examiner, teaches that "currently, the use of a retroviral transfection system is indispensable for iPS cell induction, which represents a severe disadvantage of iPS cells in terms of [a] safety issue; around 20% of iPS cell-derived mice developed tumours" and that "it may be able to induce iPS cells, with higher efficiency and without need for retroviruses." Yamanaka 55. However, this teaching does not negate or contradict the teachings of Stadtfeld and Okita; indeed, it seems implicitly to invite their teachings.

We consequently conclude that, based upon the disclosures of Appellants' Specification, which point to and explicitly incorporate, the prior art teachings of Stadtfeld and Okita with respect to the employment of adenoviral and plasmid transfection vectors, that the limitation of claim 1 reciting "an expression vector encoding at least one nuclear reprogramming factor" is enabled by Appellants' Specification. We consequently reverse the Examiner's rejection of the claims.

Because we find this argument dispositive of the appeal, we do not reach Appellants' additional argument concerning the inventor's declaration. See App. Br. 13.

DECISION

The Examiner's rejection of claims 1–5, 7, and 9–11 as unpatentable under 35 U.S.C. § 112(a) for lack of enablement is reversed.

REVERSED