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Datasheet for the decision of 22 February 2021

Case Number: T 1598/17 - 3.3.08

Application Number: 05752560.2

Publication Number: 1783205

IPC: C12N5/02, C12N5/06

Language of the proceedings: EN

Title of invention:
METHOD OF INDUCING THE DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO NERVE BY SERUM-FREE SUSPENSION CULTURE

Applicant:
Riken

Headword:
Nervous system cell/RIKEN

Relevant legal provisions:
EPC Art. 123(2), 84, 83, 54, 56

Keyword:
Main request - requirements of the EPC met (yes)

Decisions cited:

Catchword:

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Case Number: T 1598/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 22 February 2021

Appellant: (Applicant)	Riken 2-1 Hirosawa Wako-shi, Saitama 351-0198 (JP)
Representative:	Clements, Andrew Russell Niel Schlich 9 St Catherine's Road Littlehampton, West Sussex BN17 5HS (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 3 January 2017 refusing European patent application No. 05752560.2 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	R. Winkelhofer

Summary of Facts and Submissions

I. The appeal of the applicant (appellant) lies from a decision of an examining division posted on 3 January 2017, refusing the European patent application No. 05 752 560.2 with the title "Method of inducing the differentiation of embryonic stem cells into nerve by serum-free suspension culture". The application was filed under the Patent Cooperation Treaty in Japanese language. In the following, references to "the application as filed" are to the English translation documents filed on 17 January 2007.

II. In the decision under appeal, the main request then on file was found to extend beyond the content of the application as filed (Article 123(2) EPC) and lack an inventive step (Article 56 EPC), and the auxiliary request was considered not to meet the requirements of Articles 84 and 83 EPC.

III. In particular, for the assessment of inventive step the examining division regarded document (16) as the closest state of the art, and held that the sole difference between the method described therein and the method defined in claim 1 was that in the latter the cells are cultured in a non-cell-adhesive culture vessel (see sections 2.2. and 2.5 of the decision). The absence of retinoic acid and feeder cells in the medium used in the method of claim 1 for culturing the cells as a floating aggregate was not considered to be a distinguishing feature because, in the examining division's view, the first culture step described in document (16), which is carried out in a medium without retinoic acid and feeder cells, could also be regarded as a differentiation induction step (see section 2.4 of

the decision under appeal). Starting from document (16), the problem to be solved was formulated as the provision of a method to generate embryoid bodies in a serum-free medium. Since the cultivation of pluripotent cells in a non-adhesive culture vessel was known from documents (17), (6), (19) and (7), the examining division concluded that the subject-matter of claim 1 lacked an inventive step in view of document (16) "*... combined with the general knowledge of the skilled person in the art reflected in documents D6, D7 and D17*" (see section 2.7 of the decision).

IV. Together with the statement setting out the grounds of appeal, the appellant submitted three sets of claims as new main request and auxiliary requests 1 and 2, as well as documentary evidence. Oral proceedings were requested if the board did not intend to allow the appeal on the basis of the written submissions.

V. The appellant was summoned to oral proceedings. In a communication issued in preparation of the oral proceedings, the board drew the appellant's attention to some matters which appeared to be of particular significance for the decision, in particular matters in connection with Articles 123(2), 84, 83 and 56 EPC.

VI. In reply to the board's communication, the appellant submitted six sets of claims as additional auxiliary requests 3 to 8.

VII. Oral proceedings were held by videoconference on 22 February 2021. During the proceedings, the appellant submitted a set of claims that replaced the claims of the main request then on file.

VIII. Claim 1 of the new main request reads as follows:

"1. A method of inducing differentiation of a cell, which can be cultured in vitro and has a pluripotency capable of differentiating into all cells constituting the living body, into a Sox1 positive nervous system cell, which consists of culturing the cells treated for dispersion as a floating aggregate in a serum-free medium in the absence of both retinoic acid and feeder cells and in a non-cell-adhesive culture vessel."

Dependent claims 2 to 5 are directed to various embodiments of the method of claim 1.

IX. The following documents are referred to in the present decision:

(6): WO 99/32606, published on 1 July 1999;

(7): WO 03/062405 A2, published on 31 July 2003;

(16): WO 2004/007665 A2, published on 22 January 2004;

(17): WO 03/046141 A2, published on 5 June 2003;

(18): G. Bain *et al.*, 1995, Developmental Biology, Vol. 168, pages 342 to 357; and

(19): S. Rungarunlert *et al.*, 31 December 2009, World J. Stem Cells, Vol. 1, No. 1, pages 11 to 21.

X. The submissions made by the appellant, as far as they are relevant to the present decision, were essentially as follows:

Article 123(2) EPC

Claim 1 had the same wording as the corresponding claim of the auxiliary request underlying the decision under appeal, except that the word "comprises" had been replaced with "consists of" to emphasise that the differentiation induction was carried out in the absence of both retinoic acid and feeder cells. Basis for this amendment could be found in Example 1.

Article 56 EPC

The examining division erred in finding that the method of claim 1 lacked an inventive step in view of document (16) combined with the general knowledge of the skilled person. The claimed method differed from that described in document (16) in that the embryonic stem cells were differentiated into Sox1 positive nervous system cells in the absence of both retinoic acid and feeder cells in a non-cell-adhesive culture vessel. The technical effect was the provision of forebrain tissue, particularly telencephalic tissue. Starting from document (16), the technical problem to be solved was the provision of a method to generate Sox1 positive nervous system cells in the absence of both retinoic acid and feeder cells. The authors of document (16) highlighted a potential problem associated with using retinoic acid (see paragraph [00101], lines 21 and 22). To circumvent this problem, the embryonic stem cells were cultured in the presence of the PA6 stromal cell line. Hence, document (16) expressly taught that differentiation of embryonic stem cells into Sox1 positive neural cells should be carried out in the presence of either retinoic acid or feeder cells. There was no suggestion that the method should or could be modified, nor any disclosure of alternative

factors that might be useful for inducing differentiation of stem cells into neural cells in the absence of both retinoic acid and feeder cells. Thus, the claimed subject-matter involved an inventive step.

XI. The appellant requested that the decision under appeal be set aside and a patent be granted on the basis of the new main request.

Reasons for the Decision

Article 123(2) EPC

1. Claim 1 of the new main request is derived from dependent claim 9 of the application as filed which, being dependent from claim 2, includes also the features specified in claims 1 and 2. Following amendments have been introduced into the claim:

(a) The wording "*embryonic stem cells*" has been replaced by the wording "*a cell, which can be cultured in vitro and has a pluripotency capable of differentiating into all cells constituting the living body*"; a basis for this amendment is found on page 8, lines 2 to 5 of the application as filed.

(b) The feature "[differentiation] *into a Sox1 positive nervous system cell*" has been introduced to characterize the differentiated cell; this feature has a basis on page 14, lines 30 and 31 of the application as filed.

(c) The additional features "*cells treated for dispersion*" and "*in a non-cell-adhesive culture*

vessel" have a basis in the passages on, respectively, page 23, lines 19 to 31, in particular line 20; and page 22, lines 31 and 33 of the application as filed.

(d) The wording "*comprises*" has been replaced by "*consists of*".

2. The features specified in claim 2 have a basis in the passages on page 27, lines 12 to 14 and 21 to 27, and page 28, lines 8 to 15 of the application as filed. A method according to claim 3 in which Lefty-A and Dkk1 are added to the culture medium is disclosed in Example 2, in particular in the passage on page 49, lines 2 to 4 of the application as filed.

3. The features specified in claim 4 have a basis in the passages on page 29, lines 10 to 19; page 30, lines 3 to 8 and 11 to 16 (alternative (i)), and page 25, lines 19 to 29 (alternative (ii)) of the application as filed. The features in claim 5 are disclosed in the passage on page 23, lines 27 to 31.

4. Claim 6 of the main request underlying the decision under appeal, which in the examining division's view contravened Article 123(2) EPC, has been deleted in the present main request.

5. The amendments introduced into the claims do not contravene Article 123(2) EPC.

Article 84 EPC

6. The examining division did not raise any objection concerning clarity or support in the description with respect to the claims of the main request then on file.

7. Claim 1 of the present request differs from the corresponding claim of the main request underlying the decision under appeal in that the wording "*A method* [...] *which comprises* ..." has been replaced by "*A method* [...] *which consists of* ...", and that the feature "*in the absence of both retinoic acid and feeder cells*" now characterizes the medium in which the cells are cultured to induce differentiation (see section VII above). Neither clarity nor support issues arise from the introduced amendments.

8. Dependent claims 2 to 5 are, except for the deletion of "*soluble Nodal receptors and Nodal receptor*" in claim 2, identical in wording to the corresponding claims of the main request underlying the decision under appeal.

9. The board is satisfied that the requirements of Article 84 EPC are met.

Article 83 EPC

10. In the decision under appeal, an objection of lack of sufficient disclosure was raised with respect to the terms "*Nodal signal inhibitor*" and "*Wnt signal inhibitor*" without limitation to specific compounds (see section 3.5 of the decision).

11. In the present set of claims, Nodal signal inhibitors are restricted to Lefty-A, Lefty-B, Lefty-1 and Lefty-2 known in the art at the relevant date and disclosed on page 27, lines 21 to 27 of the application as filed. Wnt signal inhibitors have been limited to Dkk1, Cerberus protein, Wnt receptor inhibitors, Wnt antibodies, casein kinase inhibitors and dominant

negative Wnt proteins, as disclosed on page 28, lines 8 to 15 of the application as filed. Hence, the reasons given by the examining division for the objection do not apply to the present claims.

12. Since there are no other apparent issues which may call into question the sufficiency of the disclosure in the application as filed, the requirements of Article 83 EPC are considered to be met.

Article 54 EPC

13. No objections concerning the novelty of the claimed subject-matter were raised in the decision under appeal. As none of the documents on file forming part of the state of the art anticipates the claimed subject-matter, novelty is acknowledged.

Article 56 EPC

14. Document (16), which the examining division regarded as the closest state of the art, describes an *in vitro* method for inducing differentiation of embryonic stem cells into neural cells, in particular functional spinal motor neurons. In the method of document (16) partially dissociated embryonic stem cell colonies are grown in aggregate culture in serum-free DFK5 medium without leukemia inhibitory factor (LIF) for two days, a procedure that results in the formation of embryoid bodies (EBs) (see paragraphs [0091] and [0098], in particular the passages on page 35, lines 20 to 26 and page 38, lines 15 and 16). The culture medium is then replaced with fresh medium, and the EBs consisting initially of ~1000 cells are maintained in suspension culture for a further 1-7 days, in the presence or absence of various factors (see page 38, lines 17

to 19). Cultivation in fresh medium supplemented with retinoic acid (RA) results in the presence of many Sox1 positive cells (see page 38, lines 27 to 30).

15. The board does not share the examining division's view that the initial ES cell cultivation described on page 35, lines 20 to 26 of document (16) could be regarded as a differentiation induction step. The initial cultivation of ES cells results in the formation of floating aggregates of undifferentiated cells which are referred to as embryoid bodies (see the passage on page 38, lines 15 and 16 of document (16), as well as page 345, right-hand column, lines 4 to 8 of document (18), the latter document being cited in, *inter alia*, paragraphs [0098] and [0099] of document (16) in connection with the formation of embryoid bodies). According to document (16), differentiation of the ES cells into Sox1 positive nervous system cells is induced upon exposure of the embryoid bodies to retinoic acid (see page 38, lines 27 to 30 and second row of Figure 1B).

16. Contrary to the examination division's view, neither document (18), which is erroneously cited as document (17) in sections 2.4 and 2.7 of the decision under appeal, nor document (7) provide persuasive evidence that culturing mouse ES cells for two days in a medium as described on page 35, lines 20 to 26 of document (16) which does not contain either LIF or foetal bovine serum, but contains β-mercaptoethanol, would be sufficient to induce cell differentiation, in particular differentiation into Sox1 positive nervous system cells, as claim 1 requires.

17. It is stated in document (18) that in the presence of LIF and β-mercaptoethanol differentiation of ES cells

is prevented or suppressed (see page 343, right-hand column, last sentence under the heading "Culture of undifferentiated ES cells" and page 345, left-hand column, first sentence under the heading "RA Stimulates the Appearance of Cells with Neuron-like Morphology in ES Cultures"). However, these statements do not allow to conclude that, conversely, in the absence of LIF in the culture medium differentiation of ES cells into Sox1 positive nervous system cells is induced. Neither LIF nor a possible effect on cell differentiation are mentioned in the second paragraph of the right-hand column on page 345 of document (18), which is referred to in section 2.4 of the decision under appeal as document (17).

18. As regards document (7), it should be noted that, while differentiation of ES cells is mentioned on page 14, lines 2 to 14, the statements in this passage appear to relate to the adverse effect of LIF on the formation of embryoid bodies, rather than an effect on the actual induction of cell differentiation ("*... all of the proposed EB inducing conditions contain the step* [of] *culturing the expanded ES cells in suspension culture with a medium containing no LIF* ***to allow their aggregation*** *..."*; emphasis added by the board). Moreover, there is no indication in this passage that differentiation of ES cells in the absence of LIF would result in Sox1 positive nervous system cells, as claim 1 requires.

19. In view of the above, the board holds that document (16) does not describe or suggest any *in vitro* differentiation methods resulting in Sox1 positive nervous system cells, other than those requiring the addition of either retinoic acid (see page 38, lines 27 and 28) or feeder cells (see page 39, lines 24 to 28).

20. In the light of the results obtained in the experiments described in Examples 1 and 2 of the present application, the technical effect underlying the present invention is the efficacious induction of differentiation of ES cells into Sox1 positive nervous system cells.

21. Hence, starting from the method described in document (16), the problem to be solved is the provision of an *in vitro* method of inducing differentiation of ES cells into Sox1 positive nervous system cells in an efficient manner.

22. In view of the results in Examples 1 and 2 of the application, the board has no reason to doubt that this problem is solved by the claimed methods.

23. Unlike the examining division, the board is persuaded that, starting from the teachings of document (16) it was not obvious to a person skilled in the art to induce differentiation of ES cells into Sox1 positive nervous system cells by culturing the cells as a floating aggregate in a serum-free medium in the absence of both retinoic acid and feeder cells. It is stated in paragraph [0099] of document (16) that under control conditions, i.e. in the absence of retinoic acid:

> "... *EBs grown for 2-3 days contained few,* ***if any****, cells that expressed the pan-neural progenitor marker, Sox1 (FIG. 1B)* [...] *Similarly, EBs examined at 5 days contained few, if any, neurons.* [...] *In contrast, exposure of EBs to RA (100 nM*[sic] *to 2 µM) for 2-3 days resulted in the*

presence of many Sox1$^+$ cells (FIG. 1B)" (see page 38, lines 20 to 28; emphasis added)

24. In the light of these statements, at the relevant date a person skilled in the art had no motivation to try to efficiently induce cell differentiation into Sox1 positive nervous system cells in the absence of retinoic acid. Nor could the skilled person have any reasonable expectation of success. Hence, the method of claim 1 cannot be considered to be obvious to the skilled person.

25. In section 2.7 of the decision under appeal, passages of document (18) – erroneously cited as document (17) – as well as of documents (6), (7) and (19) – the latter being published after the filing date of the present application – were referred to as evidence that, for inducing differentiation of embryonal stem cells *in vitro*, cultivation in a non-cell-adhesive culture vessel, in particular a bacteriological dish was common general knowledge at the relevant date.

26. While this finding might be correct, the gist of the invention does not lie in a particular type of culture vessel, but rather in the teaching that *in vitro* differentiation of pluripotent cells capable of differentiating into all cells constituting the living body, e.g. embryonic stem cells into Sox1 positive nervous system cells can be induced in the absence of retinoic acid and feeder cells. The examining division failed to acknowledge this teaching and, consequently, the merit of the claimed invention.

27. For the reasons given above, the process of claim 1 involves an inventive step within the meaning of Article 56 EPC.

Conclusion

28. The requirements of the EPC are fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 5 of the main request filed during the oral proceedings before the board, and a description to be adapted.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated