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D E C I S I O N
of 3 February 2003

Case Number: T 0058/00 - 3.3.4

Application Number: 90115397.3

Publication Number: 0412557

IPC: C07K 14/00

Language of the proceedings: EN

Title of invention:
Hepatic parenchymal cell growth factor, gene encoding the same, process for producing the factor, and transformants producing the factor

Patentee:
MITSUBISHI CHEMICAL CORPORATION

Opponent:
Snow Brand Milk Products Co., Ltd.

Headword:
Hepatic growth factor/MITSUBISHI

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
"Novelty - (yes)"
"Inventive step - (yes)"
"Enabling disclosure - (yes)"

Decisions cited:
G 0009/91, G 0010/91

Catchword:
-

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Case Number: T 0058/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 3 February 2003

Appellant: (Opponent)
Snow Brand Milk Products Co., Ltd.
1-1 Naebocho 6-chome
Higashi-ku, Sapporo-shi, Hokkaido (JP)

Representative:
Wakerley, Helen Rachael
Reddie & Grose
16 Theobalds Road
London WC1X 8PL (GB)

Respondent: (Proprietor of the patent)
MITSUBISHI CHEMICAL CORPORATION
5-2, Marunouchi 2-chome
Chiyoda-ku
Tokyo (JP)

Representative:
Polz, L. Dr
Hoffmann Eitle
Patent- und Rechtsanwälte
Postfach 81 04 20
D-81904 München (DE)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 2 November 1999 rejecting the opposition filed against European patent No. 0 412 557 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: A. L. L. Marie
S. U. Hoffmann

Summary of Facts and Submissions

I. European Patent EP-0 412 557 with the title "Hepatic parenchymal cell growth factor, gene encoding the same, process for producing the factor, and transformants producing the factor" was granted on the basis of a set of 23 claims, claims 1 to 4, 22 and 23 of which read:

"1. Hepatic parenchymal cell growth factor represented by the following amino acid sequence:
Met Trp Val (... ...) Pro Gln Ser *."

"2. Hepatic parenchymal cell growth factor represented by the following amino acid sequence extending from the 30th glutamic acid to the last serine in the sequence define in claim 1:
Glu Gly Gln (... ...) Pro Gln Ser *"

"3. Hepatic parenchymal cell growth factor represented by the following amino acid sequence extending from the 32nd glutamine to the last serine in the sequence defined in claim 1:
Gln Arg Lys (... ...) Pro Gln Ser *"

"4. Hepatic parenchymal cell growth factor represented by the following amino acid sequence, wherein X denotes pyroglutamic acid:
X Arg Lys (... ...) Pro Gln Ser *"

"22. A pharmaceutical composition characterized in that it comprises a hHGF according to any of claims 1 to 4 together with a pharmaceutically acceptable diluent or excipient."

"23. The use of a hHGF according to any of claims 1 to 4 for the preparation of a pharmaceutical."

Claims 5 to 10 were directed to DNA sequences coding for the growth factors of claims 1 to 4. Claims 11 to 13 concerned various expression vectors comprising a DNA coding for said growth factor. Claims 15 to 19 were directed to processes for producing the cell growth factor, claims 20 and 21 to an animal cell transformed with expression vectors of claims 11, 12 or 13.

II. The opposition raised in view of Article 100(a) EPC for lack of novelty (Article 54 EPC) and inventive step (Article 56 EPC) and Article 100(c) for insufficiency of disclosure (Article 83 EPC) was rejected by the opposition division pursuant to Article 102(2) EPC.

III. The opponent lodged an appeal against the decision of the opposition division.

IV. The following documents are mentioned in the present decision:

(1) E. Gohda et al., Journal of Clinical Investigation, 1988, Vol. 81, pages 414 to 419

(2) E. Gohda et al., Experimental Cell Research, 1986, Vol. 166, pages 139 to 150

(3) US 5,004,805

(4) Derwent WPI Abstract Acc. No. 88-067981/198810

(5) Japanese Patent Application No. 166495/86

(E) Molecular Biology of the Cell, B. Alberts et al. editors, Garland Publishing, Inc., New York and London, 1983, pages v, 342 to 345

(G) T. Shimomura et al., Cytotechnology, 1992, Vol. 8, pages 219 to 229

(L) P. Matsudaira, The Journal of Biological Chemistry, 1987, Vol. 262, No. 21, pages 10035 to 10038

(T) T. Nakamura et al., FEBS Letters, 1987, Vol. 224, No. 2, pages 311 to 316

(Y) Declaration of Dr Naka filed on 9 July 1993 in the United States Patent and Trademark Office filed with respondent's letter of 9 September 1998

V. The Board issued a communication under Article 11(2) of the rules of procedure of the boards of appeal drawing the parties attention to the issues to be heard in the oral proceedings

VI. Oral proceedings were held on 3 February 2003, which were not attended by the appellant (cf appellant's letter of 22 January 2003).

VII. The arguments submitted in writing by the appellant (opponent) may be summarized as follows:

Article 83 EPC

- an enabling disclosure on how to make a claimed substance and how to use it based on its biological activity was required for the

patentability of the claimed substance. This was not the case of the human hepatic growth factor (hHGF) proteins of claims 1 to 4, because of the absence in the mature hHGF of the signal sequence (claim 1), the impossibility to have the forms beginning at Glu30 and Gln32, since the cleavage at and the subsequent deamination of Gln32 gave pyroglutamic acid as the N-terminal amino acid (claims 2 and 3), the single-chain structure, which was not a natural form and for the separation of which the patent in suit provided no guidance. If the HGF of claims 1 and 2 were expressed in the inclusion bodies of *E. coli*, there was no data in the patent in suit that they were still active and had their native folded structure.

Article 54 EPC

- in his statement of grounds for the appeal the appellant started his submissions with the title "I. Novelty of claims 1 to 4". However, all the arguments put forward related to enabling disclosure of the subject-matter of these claims. They are summarized above.

Article 56 EPC

- document (1), the closest prior art, disclosed the purification of hHGF from plasma of patients suffering from fulminant hepatic failure, its subunit structure and its action on the liver regeneration. The technical problem to be solved was the provision of large amounts of hHGF, this suggesting the use of genetic engineering methods.

The skilled person had a reasonable expectation of success in achieving this purpose using already well-known and routinely used methods (for instance, microsequencing, mixed oligonucleotide probes) and hence overcoming the "obstacles" defined in Dr Naka's declaration (document (Y)).

VIII. The respondent's submissions are summarized as follows:

Articles 83 EPC

- prokaryotes did not proceed eukaryotic signal sequences (document (E)), so that the expression in *E. coli* resulted in an hHGF as defined in claim 1. The hHGF of claims 2 and 3 were obtained using appropriate coding sequences or in the case of claim 3 a deaminase negative host.

- as far as the single-chain structure of the hHGF of claims 1 to 4 was concerned, document (G) showed the preparation of hHGF in a single-chain form.

Article 54 EPC

- all the arguments of the appellant under the headline "I. Novelty of claims 1 to 4" were in fact Article 83 EPC ones.

Article 56 EPC

- the appellant's comments on the obstacles mentioned in Dr Naka's declaration (document (Y)) which had to be overcome were incorrect. It was not at the priority date of the patent in suit a

matter of routine experiments to come to the solution claimed and many of the documents cited by the appellant were post-published and thus inappropriate.

IX. The appellant (opponent) requested in writing that the decision under appeal be set aside and that the European patent No. 0 412 557 be revoked.

X. The respondent (patentee) requested that the appeal be dismissed and that the patent be maintained.

Reasons for the Decision

Article 83 EPC

1. During the opposition proceedings before the first instance, the Article 83 EPC objection was raised that the final step of the purification of hHGF was insufficiently characterized insofar as it was only mentioned that this step used the reverse phase HPLC. The appellant has no longer maintained this objection in the statement of grounds for the appeal. Nevertheless, according to decision G 9/91 (EPO OJ 1993, 408) and G 10/91 (OJ 1993, 420), it is still within the framework of these appeal proceedings, since it is part of the decision of the opposition division (paragraph bridging pages 1 and 2). However, the Board considers that, at the priority date of the patent in suit, reverse phase HPLC was a well-known and commonly used method as shown for instance by documents (L) and (T). Therefore, this step fulfils the requirements of Article 83 EPC.

2. In the statement of grounds for the appeal the appellant raised under the heading "I. Novelty of claims 1 to 4" numerous new objections under Article 83 EPC (see section VI above). He argued that disclosing enabling requirements about its preparation and use was required for the patentability of a claimed substance and that the patent in suit did not give information sufficiently clear and complete to enable the skilled person to produce the hHGF of claims 1 to 4. This concerned not only the hHGF of claim 1 still mentioning the signal sequence, although upon secretion from the host cell it was cut off, but also those of claims 2 and 3, since the cleavage point of the signal sequence was Gln32, further transformed in pyroglutamic acid, so that neither hHGF with Glu30 nor HGF with Gln32 as N-terminal amino acid could be produced. Furthermore, mature hHGF was cleaved at Arg494, so that a two-chain form was obtained and not the single-chain form of claims 1 to 4. Further, since the appellant also objected to the use of hHGF of claims 1 to 4 based on its biological activity, claims 22 and 23, directed to a pharmaceutical composition containing the hHGF of claims 1 to 4 and to the use of said hHGF, respectively, were also implicitly objected to.

3. The patent in suit provides the amino acid sequences of hHGF in the single-chain form in claims 1 to 4 and Figure 1 and the corresponding nucleotide sequences in claims 8 to 10 and Figure 2. By doing so, it sets the skilled person free from slavishly reproducing the examples or methods of the patent in suit in order to come to the claimed subject-matter. On the contrary, the skilled person can now use any method being part of the common general knowledge at the priority date, such as the cell free translation system or genetic

engineering methods, which can lead to the production of the hHGFs of claims 1 to 4 in the single-chain form depending on the nucleotide sequence used. Indeed, the appellant has not shown any evidence that the expression of a sequence coding for hHGF in a host cell results in the cleavage of the single-chain form into the two-chain one. On the contrary, post-published document (G), cited as an expert opinion, shows that a specific enzyme is present in fetal calf serum used in cell culture and suggests that the cleavage does not occur in the producing cell, but in the blood, ie outside said cell. Therefore, hHGF produced in a (prokaryotic or eukaryotic) host cell is, in the absence of serum, in the single-chain form.

4. Example 3 of the patent in suit describes the expression of hHGF in B-1, B-27 and B-102 cells which are derived from CHO cells. They are first cultivated in a medium containing 10% fetal calf serum (FCS) and then during the last 72 hours in the absence of FCS. Post-published document (G) (cf supra, point 3) shows that in CHO BD-24 cells (derived from B-1, B-27 and B-102 cells) the absence of FCS leads to the formation of the hHGF single-chain form. Since hHGF produced by the CHO BD-24 cells is recovered in the culture supernatant, it has been secreted, so that the signal sequence has been cut off. Therefore, the product of Example 3 of the patent in suit is hHGF in the single-chain form, deprived of the signal sequence and having pyroglutamic acid as the N-terminal amino acid or, depending on the turn-over (ie the celerity of the degradation) of the double-chain hHGF produced during the first part of the culture in presence of FCS, at least a mixture containing hHGF under both the single-chain and the double-chain forms. Example 3 hence does

disclose the hHGF of claim 4. The skilled person was further able at the priority date to separate from each other the two forms without any burden by routine methods using the difference in the molecular weights of the two-chain and single-chain forms under reducing conditions.

5. Claims 22 and 23 of the patent in suit are directed to a pharmaceutical composition containing the hHGFs of claims 1 to 4 and to the use of said hHGFs, respectively. Example 3 of the patent in suit, as already stated above (cf supra, point 4), leads to the production of a single-chain hHGF with pyroglutamic acid as the N-terminal amino acid or to a mixture of single-chain and double-chain hHGF, which are biologically active (page 10, lines 49 and 50).

6. The appellant has not submitted evidence to the contrary, although the burden of proof laid on him.

7. It thus must be concluded that the patent in suit describes a biologically active hHGF which can be used for the preparation of a pharmaceutical composition.

8. Therefore, claims 1 to 4, 22 and 23 meet the requirements of Article 83 EPC.

Article 54 EPC

9. During the opposition proceedings, a novelty objection was raised in view of documents (1) to (5) which described the isolation of hHGF from human serum on the ground that merely defining a known protein in new terms, ie by its amino acid sequence, would not render the claimed subject-matter novel. The specific amino

acid sequence being an inherent property of the previously purified protein. The opposition division acknowledged the novelty of claim 1, because the hHGF molecules of the prior art, being extracted from serum, were deprived of the signal sequence. Novelty was also acknowledged for the subject-matter of claims 2 to 4, since the opponent failed to show that at least one of the protein bands shown in the SDS gels of document (1) corresponded to one of the proteins of claims 2 to 4.

This objection has not been maintained in the statement of grounds for appeal, but since it is part of the decision of the opposition division, it is still within the framework of the appeal proceedings (decisions G 9/91 and G 10/91, cf supra point 1). The Board, however, does not see any reason to deviate from the conclusions reached by the opposition division and acknowledges the novelty of the claims.

Article 56 EPC

10. The Board, as the appellant and the respondent, considers document (1) as being the closest prior art. Document (1) describes the four-step purification of 36.6 Fg of hHGF from 930 ml of plasma obtained from a patient suffering from fulminant hepatic failure with an overall yield of 17.8% and a purification degree of 209,000 fold (Table 1, page 416). This hHGF is further characterized by SDS-PAGE under reducing and non-reducing conditions (Figures 1 to 3) and by his stimulating effect on the proliferation of cultured hepatocytes (Table 2).

11. In view of the biological function of hHGF disclosed in document (1)(growth stimulating activity on hepatocytes), there was, at the priority date of the patent in suit, an obvious desideratum for large amounts of hHGF to be used in human medicine. Since the amount present in the plasma of patients was according to document (1) considered as being very low, this implied at that time the use of genetic engineering techniques. Consequently, the technical problem to be solved can be defined as the provision of the elements necessary for reaching said purpose.

12. The patent in suit solves this problem by providing the skilled person with the amino acid and nucleotide sequences of hHGF, expression vectors, host cells, pharmaceutical compositions and processes for the production of hHGF. Example 3 of the patent in suit shows that this problem has been successfully solved (cf supra, points 4 and 5).

13. The question for the assessment of inventive step is whether the skilled person would have straightforwardly deduced this solution from document (1), considered alone or in combination with other prior art documents or the common general knowledge and whether he/she would thereby have had a reasonable expectation of success.

14. The appellant argued that it would have only required routine experiments to determine at least a partial amino acid sequence of the purified material of document (1) using the method described in document (L) and to prepare therefrom mixed oligonucleotide probes

leading to the isolation of a sequence coding for hHGF and, finally, to its expression and use in a pharmaceutical composition.

15. The respondent stated, supported by the declaration of Dr Naka (document (Y)), that several obstacles (low concentration of hHGF in plasma, low yield of the purification procedure, impurity of the material of document (1), no known source of sequences coding for hHGF) had to be overcome.

16. In the Board's opinion, before considering the preparation of a pharmaceutical composition, the availability or the existence of a suitable cDNA library or of a source for mRNA encoding hHGF, the first aspect, with which the skilled person is confronted is the provision of an amount of hHGF sufficient in quantity and in quality (ie purity degree) for the determination of at least a partial amino acid sequence.

17. Protein sequences are determined using the Edman degradation method, identifying the constitutive amino acids one by one starting from the N-terminal end. In order to be sequenced a proteinaceous material has to have an accessible N-terminal amino acid. This is not the case of mature hHGF obtained from document (1), because of the presence of the N-terminal pyroglutamic acid. This results in the fact that the method of document (L), contrary to the appellant's suggestion, cannot be used.

18. Furthermore, the protein to be sequenced has to be pure. Impurities in relation with the Edman method may be of two kinds: proteinaceous material unrelated to

the protein to be sequenced or other molecular forms of said protein, such as degradation forms. These other molecular forms of said protein may hinder the determination of the amino acid sequence, if they do not begin at the same N-terminal point, since each cycle of the Edman degradation will identify an amino acid for each of these molecular forms.

19. The proteinaceous material of document (1) appears to be pure from unrelated proteins, but contains different molecular forms of hHGF, since with reference to Figure 3A showing the bands obtained in SDS-PAGE under non-reducing conditions, hHGF is said to consist of at least "*seven different molecular weight entities*" (page 416, left column, second paragraph). Further, on page 417 (left column, first paragraph), it is stated that "*...At least four different heavy chains appear to exist. In the case of the light chain, however, only two different size chains were detected...*". Moreover, three other bands are seen with molecular weight of 48000, 21000 and 13000 (page 416, left column, last paragraph). There is neither in document (1) nor in any of the prior art documents on file published before the priority date of the patent in suit sequence data or indication whether these various molecular entities begin at the same N-terminal amino acid. In the Board's opinion, the notional skilled person would not have considered the proteinaceous material of document (1) suitable for a determination of the amino acid sequence using the Edman method.

20. Furthermore, at the priority date of the patent in suit, the skilled person had no information about a tissue susceptible to be a potential source for hHGF mRNA or of a suitable cDNA library. The adjective

"hepatic" in the name "hHGF" only indicates the effector tissue. This is no indication that the hepatic tissue is also the producer of hHGF. On the contrary, the fact that document (1) shows that hHGF is present in the plasma, ie blood, of patient with fulminant hepatic failure is an indication that hHGF is not produced in the hepatic tissue. Indeed, one of the functions of the blood is to transport substances from their production place to their place of action. This is confirmed by document (Y) which shows that a search for a suitable tissue using oligonucleotide probes designed after partially sequencing hHGF failed and the liver was not identified as a suitable tissue. Finally, the inventors identified the placenta as a source for hHGF and this result is unexpected.

21. The Board considers that, in view of the obstacles to carry out protein sequencing to be able to prepare probes allowing the identification of a particular tissue as a source of hHGF mRNA or for the preparation or identification of a suitable cDNA library and the absence of any clue in document (1) or other document at the priority date of the patent in suit on such a tissue or library, the skilled person would have had no reasonable expectation of success and would not have embarked on what he/she would have considered as a research program with an unpredictable outcome. Therefore, claims 1 to 23 fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that

1. The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey