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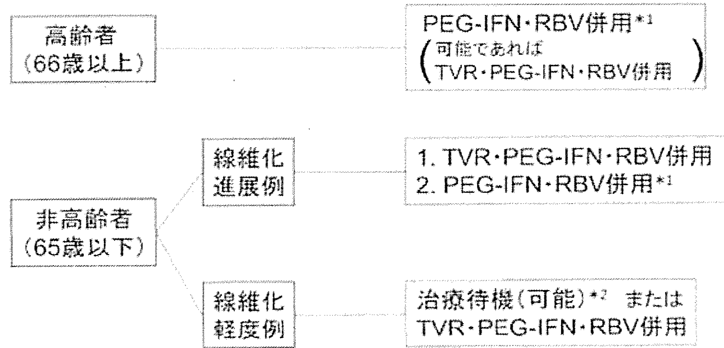
資料1　治療フローチャート

C型慢性肝炎ゲノタイプ1型・高ウイルス量症例
治療の原則(初回治療)

<IL28B SNP/core70番アミノ酸変異が測定できない場合>



*1 うつ症状合併ではIFN-β・RBV併用も考慮に入れる
*2 ALT値異常例では肝庇護療法またはPEG-IFN・IFN少量長期

C型慢性肝炎ゲノタイプ1型・高ウイルス量症例
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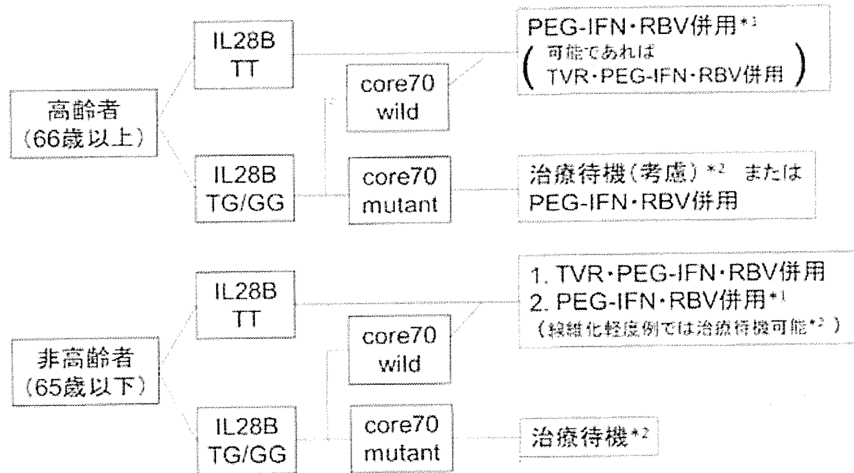

*1 うつ症状合併ではIFN-β・RBV併用も考慮に入れる
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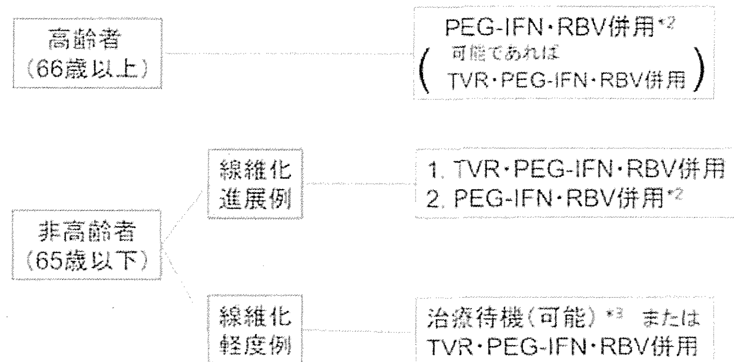
C型慢性肝炎ゲノタイプ1型・高ウイルス量症例 治療の原則（既治療）

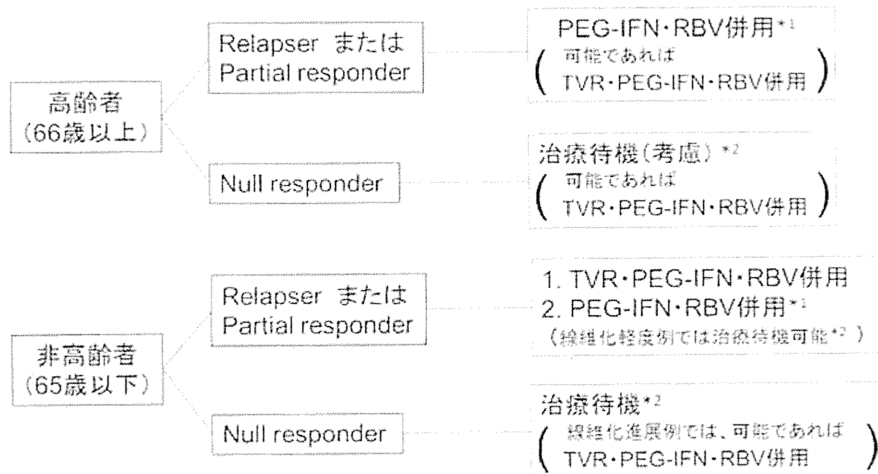
<前治療歴が不明の場合[*1]>



*1 IL28B SNP/core70番アミノ酸変異が測定可能な場合は初回治療の方針に準じる
*2 うつ症状合併ではIFN-β・RBV併用も考慮に入れる
*3 ALT値異常例では肝庇護療法またはPEG-IFN・IFN少量長期

C型慢性肝炎ゲノタイプ1型・高ウイルス量症例 治療の原則（既治療）

<前治療歴が判明している場合>



*1 うつ症状合併ではIFN-β・RBV併用も考慮に入れる
*2 ALT値異常例では肝庇護療法またはPEG-IFN・IFN少量長期

資料2　治療中止基準

(1) Peg-IFN+リバビリン併用療法の治療中止基準

HCV RNA 量低下が治療開始8週で1 log 未満、あるいは12週で2 log 未満の症例では、治療を終了することを検討すべきであり、12週で2 log 以上のHCV RNA 量低下を認めた場合も、36週までにHCV RNAの陰性化がない場合には治療を中止する。

ただし、肝細胞癌発生リスクが高く、治療開始後36週の時点でAST/ALTが正常化した症例では、治療中止基準を満たした場合でも生化学的改善効果を目指して、治療を中止せず48週までの継続治療を考慮する。

(2) テラプレビル+Peg-IFN+リバビリン併用療法の治療中止基準

治療開始4週でHCV RNA 量が 3 logcopy/ml 以下にならない症例、12週時にHCV RNAが陰性化しない症例、ならびに治療中にHCV RNA 量が 2 logcopy/ml 以上上昇する症例では、治療を中止すべきである。

(3) 生化学的改善を目指したPeg-IFN (IFN)少量投与の治療中止基準:

治療開始6か月以内にALT 値改善(40 IU/L 以下)あるいはAFP 値改善(10 ng/ml 以下)を認めない場合は治療を中止する。

資料3 ウイルス学的反応の定義

ウイルス学的反応	定義
Rapid virological response (RVR)	治療開始後4週で血中HCV RNA感度以下
extended RVR	治療開始後4週・12週のいずれにおいても血中HCV RNA感度以下
Early virological response (EVR)	cEVRあるいはpEVR
Complete EVR(cEVR)	治療開始後12週で血中HCV RNA感度以下
Partial EVR(pEVR)	治療開始後12週で血中HCV RNAが陽性だが2 log以上低下
End-of-treatment response (ETR)	治療終了時血中HCV RNA感度以下
Sustained virological response (SVR)	治療終了後24週で血中HCV RNA感度以下
Breakthrough	治療中にいったん感度以下となった血中HCV RNAが治療中に再出現
Relapse	治療中にいったん感度以下となった血中HCV RNAが治療終了後に再出現
Non-responder	治療中にHCV-RNAが感度以下にならず
Null responder	治療開始後12週で血中HCV RNAの減少が2 log未満
Partial responder	治療開始後12週で血中HCV RNAが2 log以上減少、しかし治療開始後24週で血中HCV RNAが感度以下にならない

注：AASLDから2009年に発表された「C型肝炎ガイドライン」[116]では、「治療開始後24週で血中HCV RNAが感度以下にならない」「治療開始後24週で血中HCV RNAの減少が2 log未満」「治療開始後24週で血中HCV RNAが2 log以上減少、しかし感度以下にならない」を、それぞれnonresponder、null responder、partial responderと定義していた。しかし、テラプレビルとボセプレビルの登場を期してアップデートされた2011版[101]では、nonresponderというカテゴリーは採用されず、null responder、partial responderが「治療開始後12週で血中HCV RNAの減少が2 log未満」「治療開始後12週で血中HCV RNAが2 log以上減少、しかし治療開始後24週で血中HCV RNAが感度以下にならない」と再定義されている。

本ガイドラインでは2011年版のAASLDに準じてnull/partial responderを定義し、さらにnull/partial responderを包括した"無効"として"Non-responder"を定義する。

資料4 HCVについての外注検査

IL28B SNP、HCV コア領域・NS5A 領域のアミノ酸変異は保険適用外であるものの、外注検査で測定可能である。各施設の検査会社担当者に直接照会されたい。

（1） IL28B SNP 測定

ある検査会社では、専用容器（EDTA-2Na 加）、検体量 5.0 ml、報告日数 12～16 日としている。なお、価格については各施設の検査会社担当者に直接照会されたい。

なお、IL28B 測定はヒトゲノムを検体としており、医療領域では「医療・介護関係事業者における個人情報の適切な取り扱いのためのガイドライン（厚生労働省）」、および「遺伝学的検査に関するガイドライン」（遺伝医学関連 10 学会）、「ファーマコゲノミクス検査の運用指針」（日本臨床検査医学会など）を、また研究領域では「ヒトゲノム・遺伝子解析研究に関する倫理指針」（文部科学省・厚生労働省・経済産業省）を遵守する必要がある。したがって、個人の遺伝情報の保護に十分留意しつつ、IL28B SNP 検査について患者に対して文書による説明を十分に行い、同意を得なければならない。検査会社によっては説明文書・同意書を用意しているところもあるので、参考にされたい。また、施設内に倫理委員会が設置されていれば、IL28B SNP 測定についてあらかじめ倫理委員会に申請し、承認を得るべきである。

（2） HCV コア領域・NS5A 領域のアミノ酸変異測定

ある検査会社によればそれぞれ以下のとおりである。

HCV コア領域 70 番・91 番アミノ酸変異:専用容器、検体量 5.0 ml、報告日数 10～14 日。

HCV NS5A 領域アミノ酸変異（ISDR）:専用容器、検体量 5.0 ml、報告日数 10～14 日。

なお、価格については各施設の検査会社担当者に直接照会されたい。

参考資料　平成 23 年度厚生労働省科学研究費肝炎等克服緊急対策研究事業（肝炎分野）ウイルス肝炎における最新の治療法の標準化を目指す研究班による平成 24 年 B 型 C 型慢性肝炎・肝硬変治療ガイドライン

（http://www.jsh.or.jp/medical/date/H24_guideline.pdf）

Treatment Guidelines of Hepatitis C

The Committee for Hepatitis Clinical Guidelines, Japan Society of Hepatology

Key words: hepatitis C guidelines telaprevir interferon ribavirin

Kanzo 2012; 53: 355—395

The Committee for Hepatitis Clinical Guidelines, JSH

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#Chair, The Committee for Hepatitis Clinical Guidelines

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ORIGINAL PAPER

Chimeric analysis of EGFP and DsRed2 transgenic mice demonstrates polyclonal maintenance of pancreatic acini

Je-Young Ryu · Antoni Siswanto · Kenichi Harimoto · Yoh-ichi Tagawa

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Abstract The pancreatic islet is an assembly of specific endocrine cells. There are many conflicting reports regarding whether the acinus develops from single or multiple progenitor cells. This study investigated the development and maintenance clonality of the pancreatic acinus and duct using a chimeric analysis with EGFP and DsRed2 transgenic mice. Chimeric mice (G-R mice) were obtained by the aggregation method, using 8-cell stage embryos from EGFP and DsRed2 transgenic mice. The islets from the G-R mice were chimeric and mosaic, consisting of either EGFP- or DsRed2-positive populations, as in previous reports. On the other hand, most acini developed from either EGFP or DsRed2 origin, but some were chimeric. Interestingly, these chimeric acini were clearly separated into two-color regions and were not mosaic. Some large intralobular pancreatic ducts consisting of more than 10 cells were found to be chimeric, but no small ducts made up of less than 9 cells were chimeric. Our histological observations suggest that the pancreatic acinus polyclonally and directionally is maintained by multiple progenitor cells. Pancreatic large ducts also seem to develop polyclonally and might result from the assembly of small ducts that develop from a single origin. These findings provide useful information for further understanding pancreatic maintenance.

Keywords Pancreatic maintenance · Pancreatic acinus · Chimera · EGFP · DsRed2

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Introduction

The pancreas consists of two different types of glandular tissue, exocrine and endocrine. The exocrine tissue consists of a lobulated, branched, acinar gland. The endocrine tissue is composed of compact spheroidal clusters embedded in the exocrine tissue. The acinar cells form an acinus, the functional exocrine unit that produces digestive enzymes and is connected to ducts that transport digestive secretions to the duodenum. The endocrine cells form specific regions, the islets of Langerhans, which produce pancreatic hormones. The pancreas develops from both sides of the dorsal and ventral buds in the foregut endoderm by embryonic day (E) 8.5 in mice. The two buds differentiate into exocrine or endocrine cells in the pancreas (Edlund 2001; Jørgensen et al. 2007; Slack 1995).

A useful technique in studying pancreatic organogenesis and organ maintenance involve chimeric mice that are produced by aggregating two genetically different 4 or 8-cell stage embryos. The islets of Langerhans are formed from endocrine cells that develop from multiple precursor cells (Deltour et al.

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1991; Swenson et al. 2009), and are maintained by self-duplication (Dor et al. 2004; Teta et al. 2007). Acinar precursor cells arise in the foregut endoderm at E8.5, and then differentiate into mature acinar cells to form the acinus at E12.5 (Jørgensen et al. 2007). The acini are maintained by progenitor cells differentiation (Furuyama et al. 2011). Recent reports argue that centroacinar cells are acinar progenitors (Rovira et al. 2010). There are many conflicting reports on whether the acinus develops from single or multiple progenitor cells. Some studies have suggested that acini develop from polyclonal origins (Delacour et al. 2004; Kusakabe et al. 1988), while others have argued for monoclonal origins (Dewey and Mintz 1978; Swenson et al. 2009). A report has suggested that acini develop from polyclonal origins in embryonic stage, but are maintained by monoclonal origins in adult stage (Eberhard and Jockusch 2010). All previous reports have produced chimeric mice, but they have been derived from a combination of wild-type and transgenic mice, or from mouse strains of two different genetic backgrounds with the use of antibody staining. In this study, a chimeric mouse was produced by the aggregation of two 8-cell stage embryos prepared individually from EGFP and DsRed2 transgenic mice in order to clearly show the clonality of pancreatic maintenance.

Methods

Experimental animals

EGFP or DsRed2 transgenic mice carrying the expression vectors of the enhanced green fluorescent protein (EGFP) or DsRed2 fluorescence protein genes, controlled by the CAG promoter (Kanegae et al. 1995; Niwa et al. 1991), were originally obtained via EGFP- or DsRed2-expressing embryonic stem (ES) cell—contributing chimera mice produced by the aggregation chimera method. The F1 offspring expressing EGFP or DsRed2 proteins had been backcrossed with C57BL/6JJcl or BALB/cAJcl mice. The EGFP transgenic mice were backcrossed with BALB/cAJcl to 5 generations or with C57BL/6JJcl to 6 generations. The DsRed2 transgenic mice were backcrossed with C57BL/6JJcl or BALB/cAJcl mice to 20 generations. C57BL/6JJcl and BALB/cAJcl mice were purchased from CLEA Japan, Inc. (Tokyo, Japan).

Chimeric mice were produced by the aggregation method of two genetically different 8-cell stage embryos that were prepared from EGFP and DsRed2 transgenic mice. The embryos were collected by flushing the oviducts of 2.5 days post-coitum female mice with PB1 medium. The zona pellucida was removed from 8-cell stage embryos with Tyrode's solution (Sigma-Aldrich, MO, USA). Two EGFP and DsRed2 transgenic embryos had been cultured overnight at 37 °C in a humidified 5 % CO_2 atmosphere. Blastocysts were implanted into the uteruses of 2.5 days post-coitum pseudopregnant female ICR mice. Chimeric mice were born 17 days after the implantation. The chimeric population could be identified based on coat colors, i.e., white for BALB/cAJcl and black for C57BL/6JJcl, and also based on the fluorescence of EGFP and DsRed2.

The animal protocol was approved by the Animal Experimentation Committees of Tokyo Institute of Technology.

Fluorescence imaging of mice and their organs

Fluorescence imaging of mice and their organs were observed using a fluorescence stereomicroscope outfitted with a charge-coupled device (CCD) camera (Olympus, Tokyo, Japan).

Tissue sampling, fixation, and processing

Chimeric mice were used for analysis of the pancreas at 3–8 months of age and of the small intestine at 1 week of age. BALB/cAJcl mice were used for analysis of the pancreas at 7 weeks of age. Pancreases from the mice were fixed in 10-N mild formalin (Wako, Osaka, Japan), embedded in paraffin, and sliced in 4-μm sections (Bozo Research Center, Tokyo, Japan). Small intestine from the mouse were fixed in as above, dehydrated in 30 % sucrose by overnight incubation, then quick-frozen in optimal cutting temperature compound, and sliced in 10-μm frozen sections.

Immunohistochemistry

Paraffin-embedded sections on glass slides were heated at 60 °C for 1 min, deparaffinized in xylene, and rehydrated through graded alcohols to Milli-Q

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water. The sections were washed with phosphate-buffered saline (PBS) three times and then incubated in Blocking One (Nacalai Tesque, Kyoto, Japan) for 30 min at room temperature. The chimeric pancreatic section slides were subsequently incubated with primary antibodies, including rabbit anti-red fluorescent protein (1:500; Medical and Biological Laboratories, Nagoya, Japan), and goat anti-green fluorescent protein (1:1000; Abcam, Tokyo, Japan) polyclonal antibodies with or without a mouse anti-amylase (1:50; Santa cruz biotechnology, California) antibody, for 1.5 h at room temperature. The sections were then incubated with donkey anti-rabbit IgG conjugated with Alexa Fluor 594 (1:2000; Invitrogen, NY, USA), donkey anti-goat IgG conjugated with Alexa Fluor 488 (1:2000; Invitrogen) and either 4′, 6-diamidino-2′-phenylindole dihydrochloride (DAPI) or goat anti-mouse IgG conjugated with Alexa Fluor 350 for 1 h at room temperature. The normal pancreatic section slides were subsequently incubated with a rabbit anti-laminin (1:200; Abcam) polyclonal antibody for 2 h at room temperature. Then, the slides incubated with donkey anti-rabbit IgG conjugated with Alexa Fluor 488 (1:2000; Invitrogen). Frozen sections were

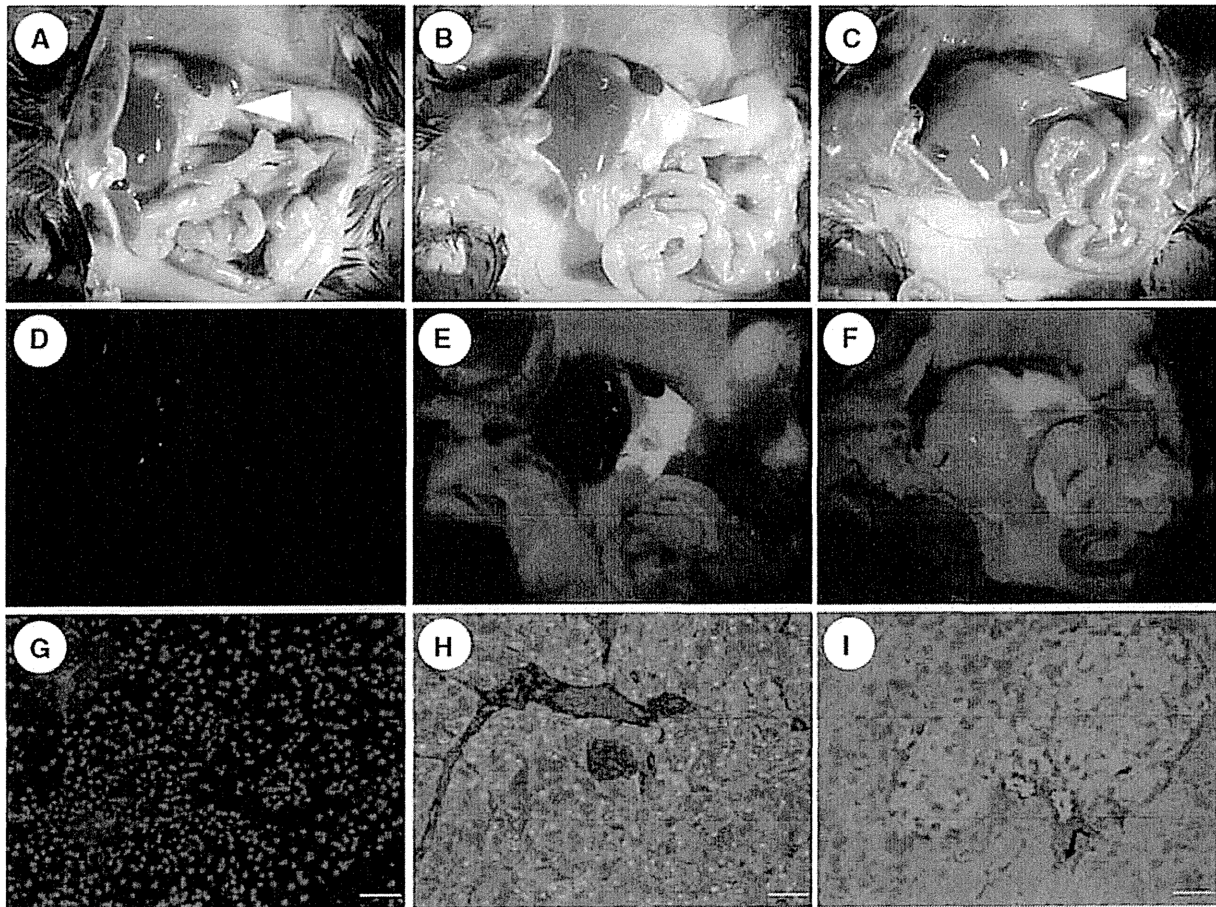

Fig. 1 EGFP and DsRed2 transgenic mice and their pancreases. **a–c** Bright-field phase image of the pancreas and other organs within the abdominal cavity viewed ventro-dorsally. **d–f** Composite overlay of DsRed2 (exposure time, 0.01 s) and EGFP (exposure time, 0.2 s) fluorescence images of the mice. **a** and **d**, an 8-week-old wild-type mouse; **b** and **e**, an 8-week-old EGFP heterozygous transgenic mouse; **c** and **f**, a 6-week-old DsRed2 homozygous transgenic mouse. These images were taken on the same day. Pancreases (*arrowheads*) in the transgenic mice were brighter than other organs. **g–i** Composite overlay of EGFP, DsRed2, and DAPI fluorescence images of pancreatic sections. **g** the section from a wild-type mouse; **h** from an EGFP transgenic mouse; **i** from a DsRed2 transgenic mouse. *Scale bars* 100 μm

incubated with DAPI. The sections were mounted in Prolong Gold fluorescent mounting medium (Invitrogen).

Imaging was performed on a fluorescence upright microscope outfitted with a CCD camera (Olympus). Images were captured using the appropriate light absorption and emission filters supplied by the manufacturer of the microscope.

Results

Production of two-color chimeric mice by the aggregation of EGFP and DsRed2 8-cell stage embryos

The 8-cell stage embryos were independently prepared from EGFP and DsRed2 transgenic mice which

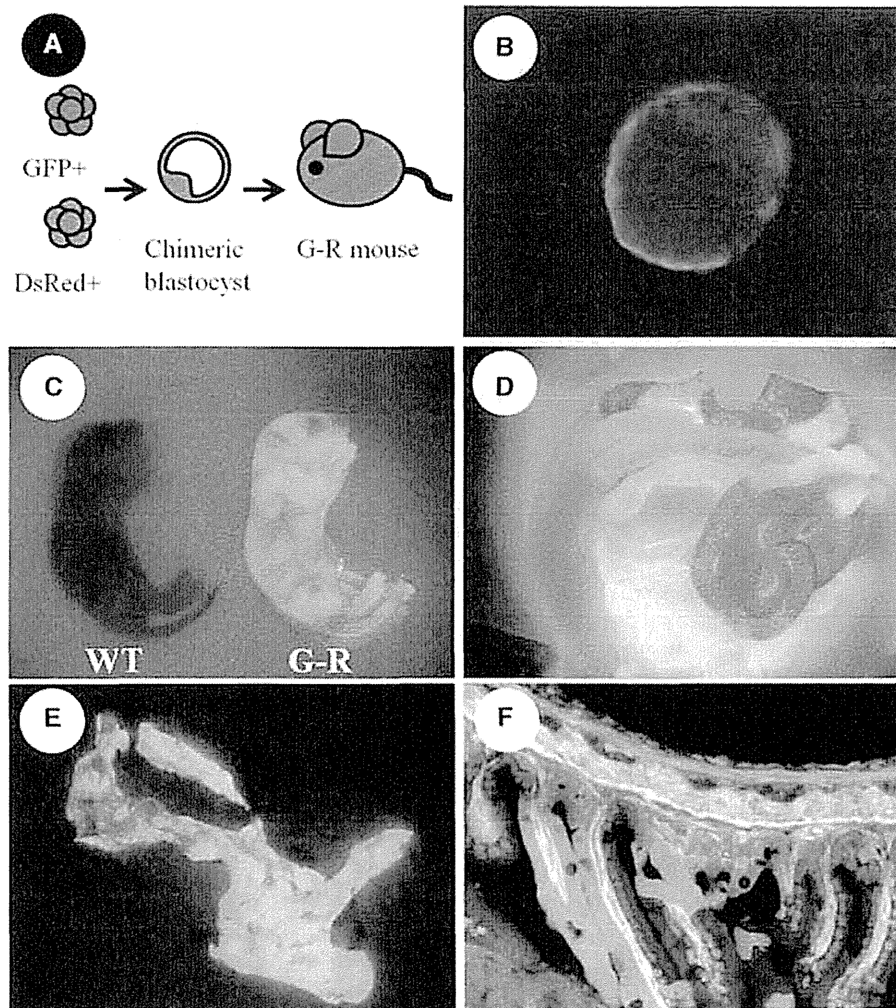

Fig. 2 Production of the G-R mice by the aggregation method. **a** G-R mice were produced by the aggregation method, using two genotypes of 8-cell stage embryos prepared individually from EGFP and DsRed2 transgenic mice. **b** Composite overlay of DsRed2 and EGFP fluorescence, and bright-field images of a chimeric embryo. This *image* was confirmed by a phase-contrast microscope. **c** Composite overlay of DsRed2 and EGFP fluorescence images of newborn mice. *Left* wild-type C57BL/6J; *Right* G-R. **d** Composite overlay of DsRed2 and EGFP fluorescence images of the 8-month-old G-R mouse showing organs within the abdominal cavity viewed ventro-dorsally. **e** Composite overlay of DsRed2 and EGFP fluorescence images of the pancreas of the G-R mouse. **f** Composite overlay of DAPI, DsRed2, and EGFP fluorescence images of the small intestine of a 1-week-old G-R mouse section. *Scale bars*, 100 μm

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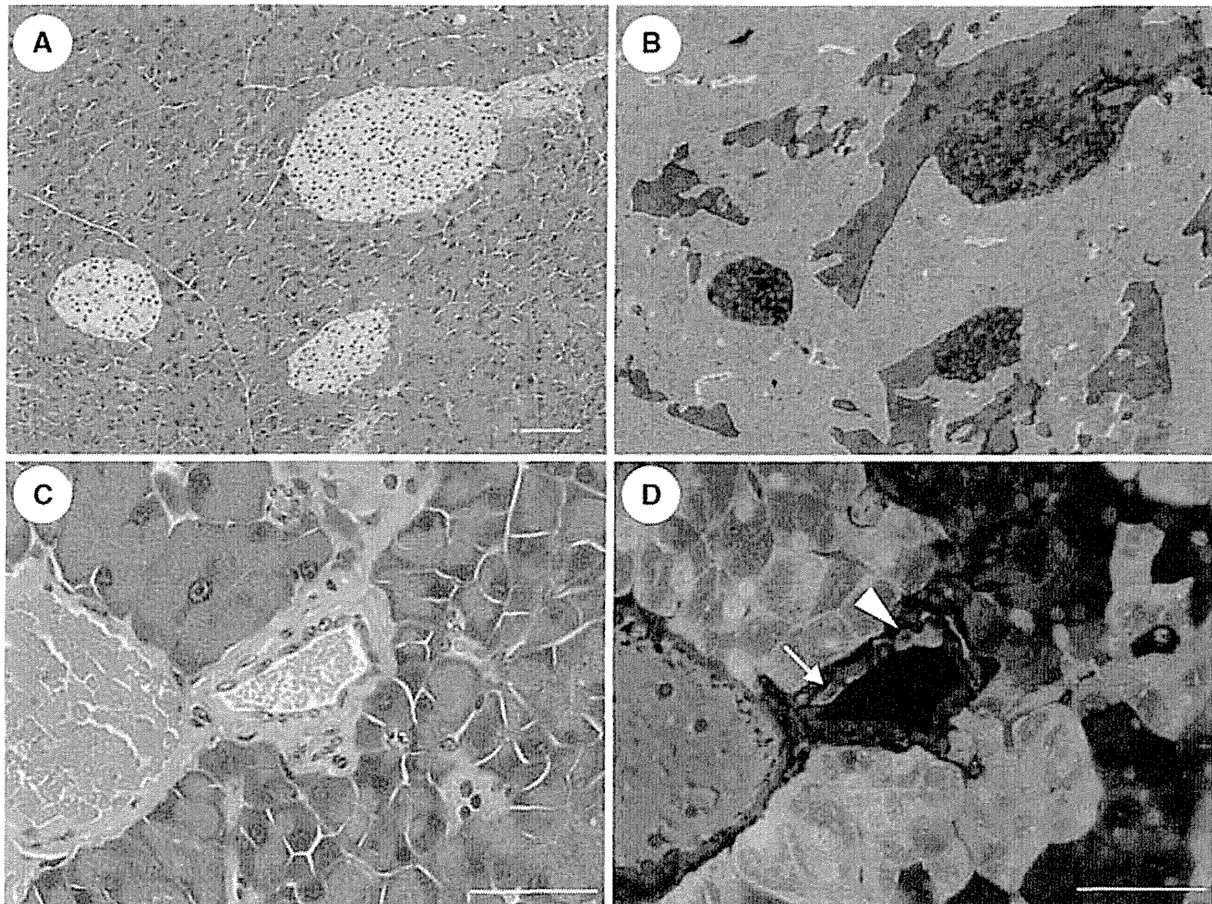

Fig. 3 Pancreatic islets and ducts were formed from polyclonal origin. **a** and **c** Images of the hematoxylin—eosin stained serial sections of the 8-month-old G-R mouse. **b** and **d** Composite overlay of DAPI, DsRed2, and EGFP images of the sections. In **d**, an EGFP-positive cell (*arrow*) and a DsRed2-positive cell (*arrowhead*) are included in the duct. *Scale bars* **a** 100 μm; **c** and **d** 50 μm

Table 1 Chimerism of pancreatic structures

Structure	No. of counts	Chimeras	Patterns
Islet	21	20	Mosaic
Acinus	112	6	Not mosaic
Large duct	20	7	Mosaic
Small duct	21	0	–

are produced by an EGFP- or a DsRed2-expressing ES cell line. These mice have green or red fluorescence expression in whole organs and tissues, in particular, very strong fluorescence signals in their pancreases (Fig. 1). As can be seen in Fig. 2a, using the aggregation procedure two different embryos were aggregated together after removing their zonae pellucidae, and cultured to form a single chimeric blastocyst (Fig. 2b). Ninety-five chimeric blastocysts were transferred into the uteruses of pseudopregnant female mice. Twenty-six mice were born, six of which were obviously chimeras based on the fluorescence of both EGFP and DsRed2, i.e., G-R mice, shown in Fig. 2c. All of the internal organs of the G-R mice, including pancreas, also demonstrated either green or red fluorescence, as shown in Fig. 2d and e.

Analysis of the clonality and development of the pancreatic acini, ducts, and islets in the chimeric mice

The formalin-fixed pancreases which outwardly demonstrated EGFP- and DsRed2-positive chimerism

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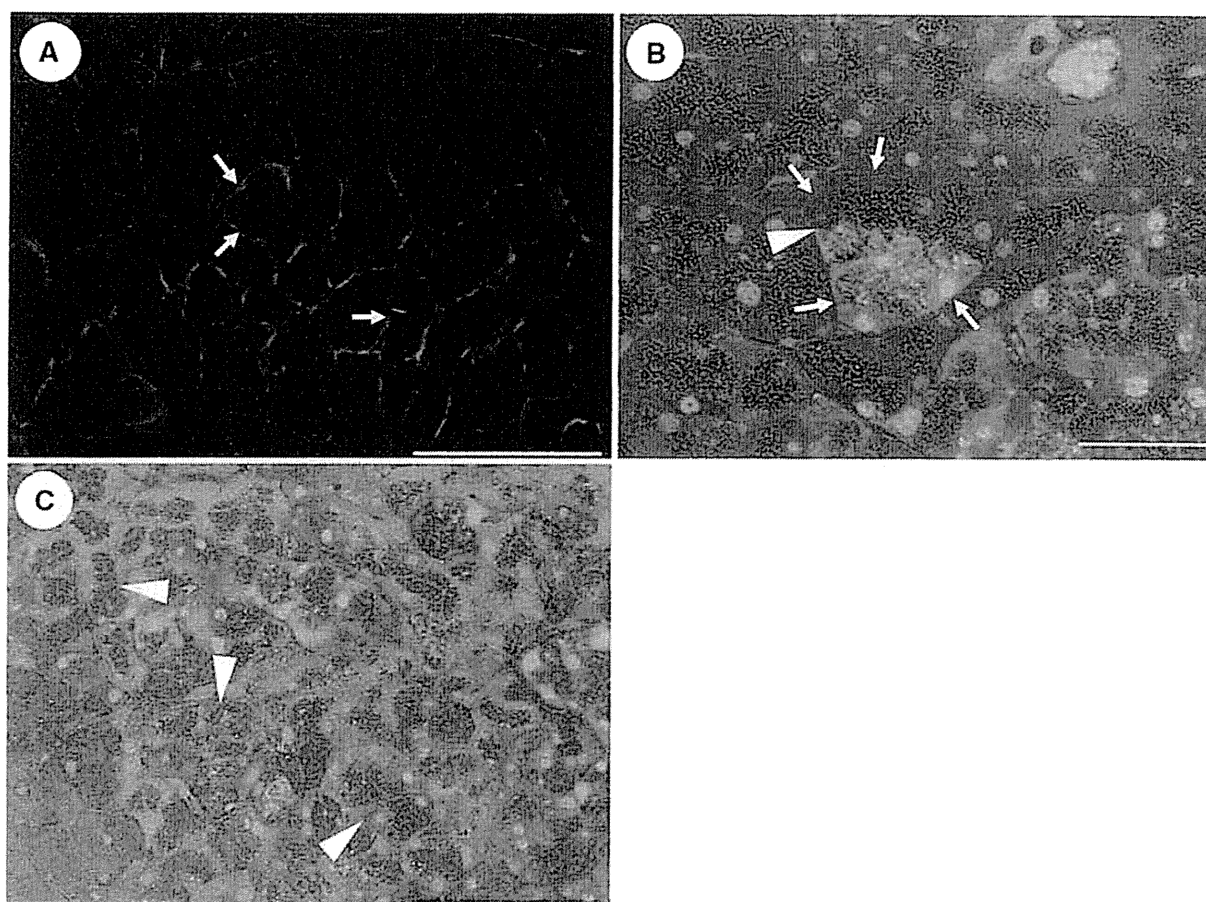

Fig. 4 Chimeric acini are clearly separated into two-color regions, and are not mosaic. **a** Composite overlay of bright field and laminin fluorescence images of the 7-week-old BALB/cA mouse section. *Arrows* point to basement membrane (laminin). **b** Composite overlay of bright field, DAPI, DsRed2, and EGFP fluorescence images of the 3-month-old G-R mouse section. A chimeric acinus (*arrowhead*) is surrounded by basement membrane (*arrows*) and no basement membrane between an EGFP positive cell and a DsRed2 positive cell. **c** Composite overlay of amylase, bright field, DsRed2, and EGFP fluorescence images of the section. *Arrowheads* point to chimeric acini. *Scale bars* 100 μm

(Fig. 2e) of the G-R mice were sliced for histological examinations involving hematoxylin—eosin staining (Fig. 3a, c) and immunological staining using anti-GFP and anti-DsRed antibodies and DAPI (Fig. 3b, d). Histological examination of these sections revealed that they were also chimeric, as shown in Fig. 3b. The eosin-positive (pink-colored) particles in Figs. 3a and c were mainly zymogen granules. The endocrine cells in the islets of Langerhans (Fig. 3a) were chimeric and mosaic, positive for either EGFP or DsRed2 (Fig. 3b). We found that 20 of 21 islets in a pancreas section of a G-R mouse were chimeric (Table 1), and all of the chimeric islets were clearly mosaic. Of 20 large ducts from the G-R mouse pancreatic section (Figs. 3b, d), seven were chimeric (Table 1). However, of 21 small intralobular ducts containing 3–9 cells each, none were chimeric (Table 1).

Next, focusing on the pancreatic acini, we prepared serial sections of the G-R mouse pancreas. We identified the acinar boundaries by bright-field observation of basement membrane (Fig. 4a) and distribution of zymogen granules. There were either EGFP- or DsRed2-positive cells in the most of the acini, but some acini consisted of both EGFP- and DsRed2-positive cells as can be seen in Fig. 4b and c, suggesting that an acinus is not monoclonally maintained from a single acinar progenitor cell. Interestingly, these chimeric acini were not mosaic, but were clearly

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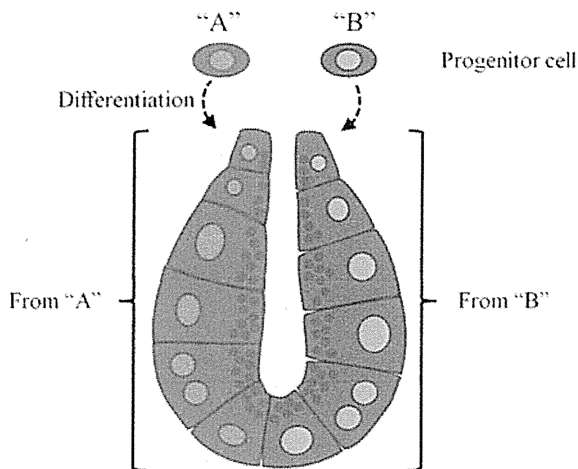

Fig. 5 A model of the pancreatic acinus maintenance. An acinus is polyclonally maintained from progenitor cells. The acinus consisted of acinar cells, which are derived from progenitors, and separated into two regions

separated into two color regions as shown in Fig. 4b and c. We found that 6 of 112 acini in a section from a G-R mouse pancreas were chimeric, and all of the chimeric acini contained clearly separated color regions and were not mosaic, as shown in Fig. 4b and c and Table 1.

Discussion

Chimeric mice have proved useful in the investigation of organogenesis and organ maintenance in various systems, for example, the intestinal crypt (Ponder et al. 1985; Schmidt et al. 1985), adrenal cortex (Iannaccone et al. 2003), and cerebellar cortex (Oster-Granite and Gearhart 1981). The method used in this study to produce chimeric mice involves the aggregation of two genetically different early embryos, such as those from two different genetic backgrounds, C57BL/6JLac and DDK, C57BL/6JLac and CBA/CaLac, BALB/cBy and C57BL/6J, or wild-type and reporter gene transgenic mice. However, several studies have produced conflicting results regarding pancreatic development and maintenance. These reports used C3H/HeN and BALB/c (Kusakabe et al. 1988), C3H/HeN and C57BL/10 (Kusakabe et al. 1988), XX and XY (Swenson et al. 2009), and wild-type and EGFP transgenic mice (Eberhard and Jockusch 2010). In antibody experiments, the question must always be raised whether negative cells represent true negatives or failed interactions, and whether positive cells represent true or false positives. In using transgenic mice, the studies mentioned above used a wild-type mouse as a counterpart for the aggregation chimera. In this study, we used the aggregation method to produce chimeric mice using two genotypes of EGFP and DsRed2 transgenic mice, and could clearly show that the pancreatic acinus is maintained from multiple progenitor cells. The small intestinal crypts were populated exclusively by DsRed2 or EGFP positive cells (Fig. 2f). The clonality of the crypts is consistent with previous findings. Our approach is validated to show that clonality of tissues. Mutant DsRed transgenic mice have not been popular because it was believed to be difficult to obtain widespread expression of DsRed due to its cytotoxic effects (Hadjantonakis et al. 2002). However, Nagy succeeded in producing the DsRed.T3 transgenic mouse (Vintersten et al. 2004). We have also independently produced a DsRed2 transgenic mouse (Figs. 1c, f). This DsRed2 transgenic mouse exhibits very strong red fluorescence in the pancreas without the need for an excitation light source, therefore this mouse confers an advantage for the investigation of pancreatic development and maintenance. In the pancreas of our EGFP transgenic mouse, the green color could be observed even in daylight (Fig. 1b, e).

In this study, we used G-R mice to demonstrate that islets of Langerhans, acini, and ducts developed from single or polyclonal origins in the pancreas. Certainly, even as almost all pancreatic cells in our transgenic or chimeric mice expressed the EGFP or DsRed2 (Figs. 1h, i, 3b), some cells no expressed neither EGFP or DsRed2 as can be seen in Fig. 3d. However, if islets, acini, or ducts containing both the cells expressing EGFP and DsRed2, we can conclude that the islets, acini, or ducts are polyclonal. Our result showing that the islets of Langerhans were chimeric and mosaic (Fig. 3b) suggests that these structures develop from polyclonal precursor cells. This conclusion agrees with those of previous reports (Deltour et al. 1991; Swenson et al. 2009). On the other hand, there have been conflicting results with respect to pancreatic acini (Swenson et al. 2009; Dewey and Mintz 1978). We demonstrated that some acini consisted of both EGFP- and DsRed2-positive cells and clearly separated into two-color regions, suggesting that an acinus is polyclonally maintained from progenitor cells and this maintenance has directional properties such as those

seen in small intestinal villus maintenance (Wice and Gordon 1998). According to our observations, the acinar maintenance pattern is displayed as in Fig. 5. The chimeric structure was also observed in the large intralobular ducts but not in the small ducts. Although this result likely reflects pancreatic ductal cell branching morphogenesis, it suggests that the large ducts are formed by assembly of small ducts that are monoclonal in origin. However, more observations are required to confirm whether the small duct is maintained monoclonally or polyclonally.

G-R mice could be useful for other studies, such as those investigating the lineages of the intestinal crypt and villus in the small intestine, the skeletal muscle in the leg, the hepatocyte in the hepatic acinus, and the cardiac muscle in the heart. By using G-R mice, the crypt, villus, and skeletal muscle lineages can be characterized more clearly, and those of hepatocytes and cardiac muscle can be investigated with more precision.

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