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REGULAR ARTICLE

Glycine regulates proliferation and differentiation of salivary-gland-derived progenitor cells

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Abstract Amino acids have various physiological activities that influence processes such as intestinal regeneration, EGF secretion, protein synthesis, and cell growth. Salivary glands are exposed to nutrients that influence their proliferation and regeneration. Glycine is included in saliva in large quantities and reportedly has important roles in antibacterial activities and the inhibition of tumor growth and as a precursor of nucleotide synthesis in cell proliferation. We have investigated the effects of glycine on the proliferation and differentiation of salivary glands by using mouse salivary-gland-derived progenitor (mSGP) cells. In cultures of mSGP cells, cell proliferation is suppressed in the presence of glycine, whereas it is promoted by its removal. Glycine promotes three-dimensional formations of mSGP cells, which are negative for immature markers and positive for differentiation markers. In cell-cycle analysis, cell-cycle progression is delayed at the S-phase by glycine supplementation. Glycine also suppresses the phosphorylation of p42/p44MAPK. These results suggest that glycine suppresses the proliferation and promotes the differentiation of mSGP cells, and that it has inhibitory effects on growth factor signaling and cell-cycle progression. Glycine might therefore be a physiological activator that regulates the proliferation and differentiation of salivary glands.

Keywords Salivary glands · Glycine · Growth factor · Mouse salivary-gland-derived progenitor (mSGP) cells · Cytokeratin (CK) 19 · Cell cycle

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Introduction

The major components of the gastrointestinal system are the oral cavity, salivary glands, esophagus, stomach, intestine, liver, and pancreas. Since the gastrointestinal tract is directly exposed to external nutrients, it is the component that is the most affected by them. For example, the intestinal epithelium contains subpopulations of cells that exhibit the most rapid turnover and synthesis of proteins (Drucker et al. 1996). The supply of most of the amino acids in the intestine is highly dependent on interstitial absorption. In particular, L-glutamate, L-glutamine, and L-aspartic acid are taken up as an energy source directly from the lumen. In addition, there are numerous reports of the role of L-glutamine as a biologically active factor in the salivary gland and the intestinal epithelium in which it stimulates epidermal growth factor (EGF) secretion, accelerates intestinal immunity by the secretion of EGF, and induces intestinal epithelial regeneration (Wilmore et al.

1988). Therefore, these amino acids may regulate the functions of various organs.

Amino acids are known to have various physiological activities depending on the biological conditions. For example, branched-chain amino acids (BCAAs) such as L-valine, L-leucine, and L-isoleucine have physiological properties such as accelerating protein synthesis and inhibiting protein degradation in skeletal muscle (Goldberg and Chang 1978). BCAAs, which account for approximately 40% of all the free amino acids in serum, are useful energy sources during exercise and for maintaining blood-sugar levels, as mediated by the glucose-alanine cycle (Harper et al. 1984). L-arginine is an intermediate in the urea cycle and is also a precursor for the synthesis of nitric oxide and polyamines (Peranzoni et al. 2007; Rees et al. 1998; Reynolds et al. 1990). L-arginine is known to stimulate the secretion of various hormones (Ghigo et al. 1994). Moreover, L-arginine deprivation can cause a delay in cell-cycle progression in HeLa cells (Wheatley et al. 2000). A high-dose of L-alanine has been reported to improve liver function and the survival ratio in rats with acute liver failure caused by a lethal dose of D-galactosamine (Maezono et al. 1996). Several other amino acids are thought to be vital for cell proliferation and important for maintaining homeostasis as neurotransmitters and as a source of adenosine triphosphate. Glycine is a component of natural products and functions as an inhibitory neurotransmitter (Ghavanini et al. 2005). Glycine also exists in saliva, inhibiting the co-agglutination of oral bacteria (Amano et al. 1997). In addition, glycine prevents tumor growth in vivo (Rose et al. 1999a, 1999b).

In this study, we have analyzed the effect of glycine on cell proliferation and differentiation by using mouse salivary-gland-derived progenitor (mSGP) cells. The salivary glands originate from the endoderm and the ectoderm, both of which participate in organogenesis (Larsen et al. 2001; Denny et al. 1997). mSGP cells were originally isolated from c-kit$^+$/Sca-1$^+$ small epithelial ducts that had proliferated and been detected by a fluorescence-activated cell sorter (FACS) only after duct ligation (Hisatomi et al. 2004; Matsumoto et al. 2007; Okumura et al. 2003; Sato et al. 2007). Isolated immature mSGP cells, which differentiate into hepatic and pancreatic cells, are positive for c-kit, Sca-1, Thy-1, intracellular laminin, CD49f, and alpha-fetoprotein (AFP). These cells are reportedly negative for differentiation markers such as cytokeratin (CK) 19, insulin, and albumin, and they do not exhibit any of the characteristics of duct epithelial cells (Hisatomi et al. 2004). mSGP cells can form cell clusters autonomously on type I collagen and differentiate at the tips of the clusters, which exhibit intracellular-laminin disappearance and CK19 expression. Therefore, since the number of cell clusters, CK19-positive cells, and intracellular laminin-negative cells reflects differentiation, we have employed a three-dimensional (3-D) culture assay to screen and detect the effect of amino acids on the differentiation of mSGP cells.

Materials and methods

Cell isolation and culture

Cells were isolated and cultured as previously described (Hisatomi et al. 2004). Cells isolated from submandibular glands, named mSGP cells, were plated and cultured on type I collagen (Asahi Techno Glass, Tokyo, Japan) at a density of 1×10^6 cells/100-mm dish, in control culture medium. The medium was renewed every 3 days.

The control culture medium was Williams' medium E supplemented with 5% fetal bovine serum (Invitrogen), 20 ng/ml mouse EGF (Chemicon International), 10^{-6} mol/l dexamethasone (Sigma, St Louis, MO., USA), 100 U/ml penicillin G, 100 μg/ml streptomycin (Invitrogen), 1× insulin-transferrin-serenium-X (Invitrogen), and 10 mmol/l nicotinamide (Sigma).

Preparation of media with or without amino acids

Delta media (δ media) were provided by AJINOMOTO (Kawasaki, Japan): the 20 types of δ media consisted of the 20 kinds of amino acids with the exclusion of one amino acid (0 mM) each. The media were prepared based on an amino-acid-free medium of the same composition as Dulbecco's modified Eagle's medium (DMEM) except for the amino acids. The final concentration of amino acids in these media was based on that of DMEM as follows (in mM): 0.4 mM glycine, 0.4 mM L-alanine, 0.4 mM L-arginine, 0.4 mM L-asparagine, 0.4 mM L-aspartic acid, 0.2 mM L-cystine, 4.0 mM L-glutamine, 0.4 mM L-glutamic acid, 0.2 mM L-histidine, 0.8 mM L-isoleucine, 0.8 mM L-leucine, 0.8 mM L-lysine, 0.2 mM L-methionine, 0.4 mM L-phenylalanine, 0.4 mM L-proline, 0.4 mM L-serine, 0.8 mM L-threonine, 0.08 mM L-tryptophan, 0.4 mM L-tyrosine, and 0.8 mM L-valine.

Medium with the addition of glycine, L-alanine, or L-serine at a concentration of 10 mM was called plus (+) medium (+Gly, +Ala, or +Ser, respectively). All media were prepared 24 h before use and kept at 4°C.

In vitro cell proliferation studies

The number of proliferating cells present at 24 h and 48 h was determined by a modified 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT)-based assay (Kishida Reagents Chemicals, Osaka, Japan) and bromodeoxyuridine (BrdU) labeling as in the manufacturer's protocol.

In vitro cell differentiation studies

To induce cell differentiation, mSGP cells were cultured on type I collagen (Asahi Techno Glass) at a density of 2×10^5 cells/60-mm dish in +Gly, +Ala, or +Ser for 2 weeks. The medium was renewed every 3 days. After 2 weeks, the phenotypes of the differentiated cells cultured in these media were estimated by immunofluorescent stains and reverse-transcriptase polymerase chain reactions (RT-PCR).

In vitro cell signal inhibition studies

To inhibit cell proliferation signaling, mSGP cells were cultured in media supplemented with a 5 μM MEK inhibitor (U0126), 20 μM PI3K inhibitor (LY294112), 20 μM Akt inhibitor (Calbiochem Biochemicals, Darmstadt, Germany), or 5 nM rapamycin (Cell Signaling Technology, Danvers, Mass., USA) for 24 h. The cell phenotypes were then estimated, as was cell differentiation.

Analysis for DNA content, cyclin expression, and apoptosis

Cells were plated on type I collagen (Asahi Techno Glass) at a density of 2.5×10^5 cells/60-mm dish and treated with 10 mM glycine for 24 h. The DNA content, cyclin expression, and apoptosis of cells treated with glycine were analyzed by flow cytometry on a Becton Dickinson FACS Caliber (BD Bioscience), as in a previous study (Hisatomi et al. 2004).

Immunostaining of cultured cells

Cells on glass dishes were stained as previously described (Hisatomi et al. 2004). The primary antibodies used were anti-CD49f (BD Bioscience PharMingen), anti-AFP, anti-CK19, anti-E cadherin (Santa Cruz Biotechnology), anti-laminin, or anti-albumin (DAKO Cytomation) diluted at 1:100 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin, for 1 h at 37°C. The secondary antibodies used were Alexa488-labeled anti-goat IgG, Alexa488-labeled anti-rabbit IgG, Alexa594-labeled anti-mouse IgG, Alexa594-labeled anti-goat IgG, Alexa594-labeled anti-rat IgG, Alexa594-labeled anti-rabbit IgG (Molecular Probes) diluted at 1:1,000 in PBS containing 1% bovine serum albumin, for 1 h at 37°C. Cells were viewed under a confocal laser-scanning microscope FV500 (Olympus Optical, Tokyo, Japan).

RT-PCR analysis

Total RNA isolation and complementary DNA preparation were as previously described (Hisatomi et al. 2004; Matsumoto et al. 2007; Sato et al. 2007). The resulting complementary DNA was amplified by using GeneAmp PCR 9700 (Perkin-Elmer, Norwalk, Conn., USA) with the following sets of primers: AFP: forward 5′-actcaccc caaccttcctgtc-3′, reverse 5′-cagcagtggctgataccagag-3′; albumin: forward 5′-catgacaccatgcctgctgat-3′, reverse 5′-ctctgatcttcaggaagtgtac-3′; CK19: forward 5′-gtcctacagattga caatgc-3′, reverse 5′-cacgctctggatctgtgacag-3′; GAPDH (D-glyceraldehyde-3-phosphate dehydrogenase): forward 5-ccgccaccaccaactgctta-3′, reverse 5′-tcatgagcccttccacaatg-3′.

Western blot analysis

Cells were grown in culture in the presence of 10 mM glycine or inhibitors or in control medium for the time periods indicated. The cells were lysed in complete RIPA buffer (150 mM NaCl, 50 mM TRIS-HCl pH 7.5, 1% NP-40, 0.1% sodium deoxycholate, 1 mM EDTA) containing a protease inhibitor cocktail and phosphatase inhibitor cocktail (Nacalai Tesque). Aliquots containing 20 μg protein were loaded per lane onto 1.5-mm 10% SDS-polyacrylamide gels (ATTO, Tokyo, Japan) for electrophoresis and subsequently transferred to a polyvinylidene difluoride membrane; the expression of each protein was confirmed as previously described (Lianguzova et al. 2007). The primary antibodies used were anti-phospho-p42/p44MAPK, anti-p42/p44MAPK (Cell Signaling Technology) diluted at 1:1,000, and the secondary antibodies were horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and HRP-conjugated anti-biotin IgG (Cell Signaling Technology) diluted at 1:2,000.

Results

Short-term glycine addition inhibits cell proliferation

In order to investigate whether glycine affected cell proliferation in vitro, we utilized mSGP cells, i.e., tissue progenitor cells derived from duct-ligated submandibular glands and originating from small epithelia ducts; these cells were able to grow while maintaining their immaturity in the presence of EGF (Hisatomi et al. 2004; Matsumoto et al. 2007; Okumura et al. 2003; Sato et al. 2007). To investigate the effects of each amino acid on cell proliferation, we cultured mSGP cells in media supplemented with one of the 20 amino acids. Cell proliferation in the presence of glycine was reduced in comparison with that of the control, whereas cells treated with L-alanine or L-serine grew as well as the control population. Based on these data, we selected three amino acids, viz., glycine, L-alanine, and L-serine, in order to investigate whether these amino acids affected cell proliferation in vitro. We cultured mSGP cells for 48 h with glycine, L-alanine, or L-serine and observed

the morphology and counted the numbers of mSGP cells every 24 h by a modified MTT assay. The number of cells increased in all cultures. However, glycine suppressed the number of cells at 24 h and 48 h (Fig. 1b). In the media with L-alanine or L-serine, no changes were seen in the cell morphology or the proliferation of mSGP cells (Fig. 1a, b). To confirm the effect of glycine on cell proliferation, we used an assay with BrdU. These results also showed that glycine supplementation suppressed the numbers of BrdU-positive cells at 24 h, similar to the MTT assays (Fig. 1c, d).

Short-term glycine depletion promotes cell proliferation

A decrease in glycine might promote cell proliferation. To determine the effects of a deprivation of glycine, we cultured mSGP cells with various amino acids removed from the medium, for 48 h. We prepared 20 types of medium (δ medium), which consisted of amino acid groupings with the removal of one amino acid each. Glycine-free medium (δGly) demonstrated a promotion of proliferation compared with control at 48 h (Fig. 2b). Depletion of L-cysteine, L-glutamine, L-glutamic acid, L-aspartic acid, L-arginine, or L-proline resulted in a reduction in cell number at 48 h (Fig. 2b), as did depletion of L-threonine, L-methionine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-tryptophane, L-lysine, or L-histidine. No changes were observed in the L-alanine, L-serine, L-asparagine, or L-tyrosine depleted media compared with the control. Morphology was maintained in all of the δ media (Fig. 2a). Thus, glycine had unique effects on cell growth; glycine-free medium promoted the proliferation and glycine supplementation suppressed the proliferation of mSGP cells.

To investigate whether glycine inhibition of cell proliferation was associated with amino acid toxicity, we cultured mouse fetal fibroblasts (E13) for 48 h in 0 mM, 2.5 mM, 5 mM, 10 mM, 20 mM, or 50 mM concentrations of glycine. We counted the number of cells every 24 h by

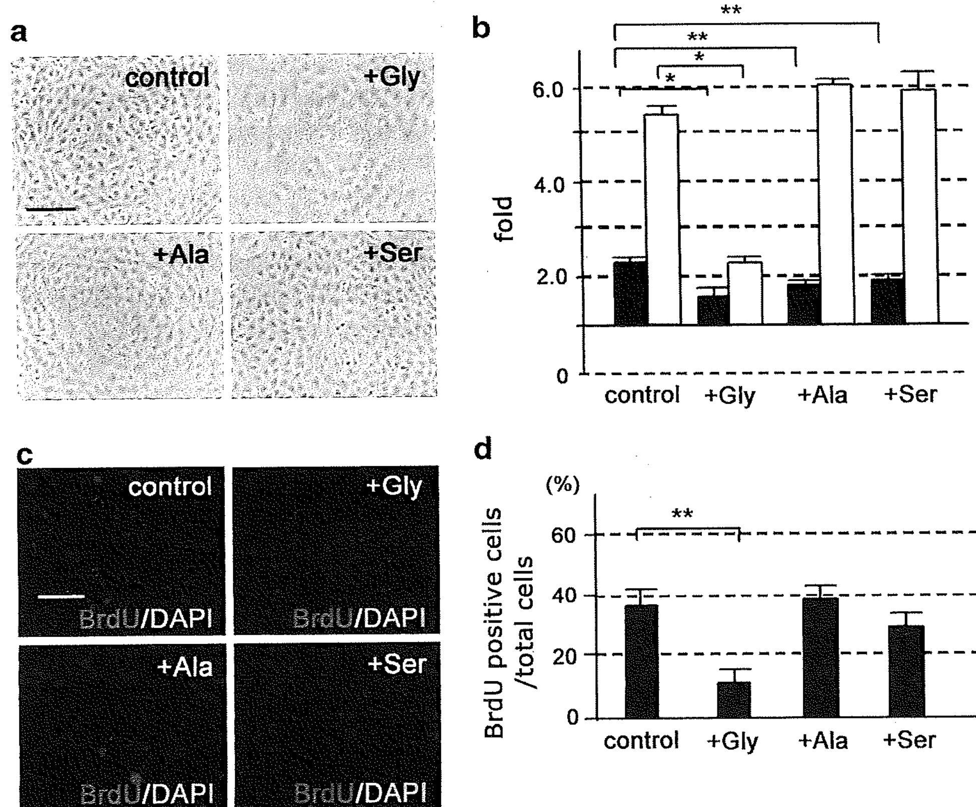


Fig. 1 Glycine inhibits cell proliferation of mSGP cells in short-term culture. mSGP cells were cultured in media supplemented with glycine, L-alanine, or L-serine. The morphology was observed, and the numbers of mSGP cells were counted every 24 h by modified MTT assay. **a** Morphology of mSGP cells at 24 h; this morphology was maintained in all cultures. *Bar* 100 μm. **b** Effects of amino acids on mSGP cell proliferation (*y-axis* numbers of proliferating cells at each time point/numbers of cells at 0 h, *black bars* 24 h, *white bars* 48 h). Glycine suppressed the numbers of cells at 24 h and 48 h (24 h: 2.23-fold in *control*, 1.19-fold in *+Gly* compared with culture at time 0 h; 48 h: 5.67-fold in *control*, 2.27-fold in *+Gly*, compared with culture at time 0 h. $^*P<0.001$, $^{**}P<0.05$). **c**, **d** Cell proliferation analysis by BrdU assay. **c** Immunofluorescent staining of mSGP cells. Glycine suppressed the numbers of BrdU-positive cells (*red*). Counterstained with 4,6-diamidino-2-phenylindole (*DAPI*). *Bar* 100 μm. **d** Quantitative analysis of BrdU-positive cells (*y-axis* numbers of BrdU-positive cells/total number of cells). The ratio of BrdU-positive cells was decreased by glycine compared with control (*control* 41.19%, *+Gly* 11.9%, *+Ala* 45.93%, *+Ser* 28.35%, $^{**}P<0.05$)

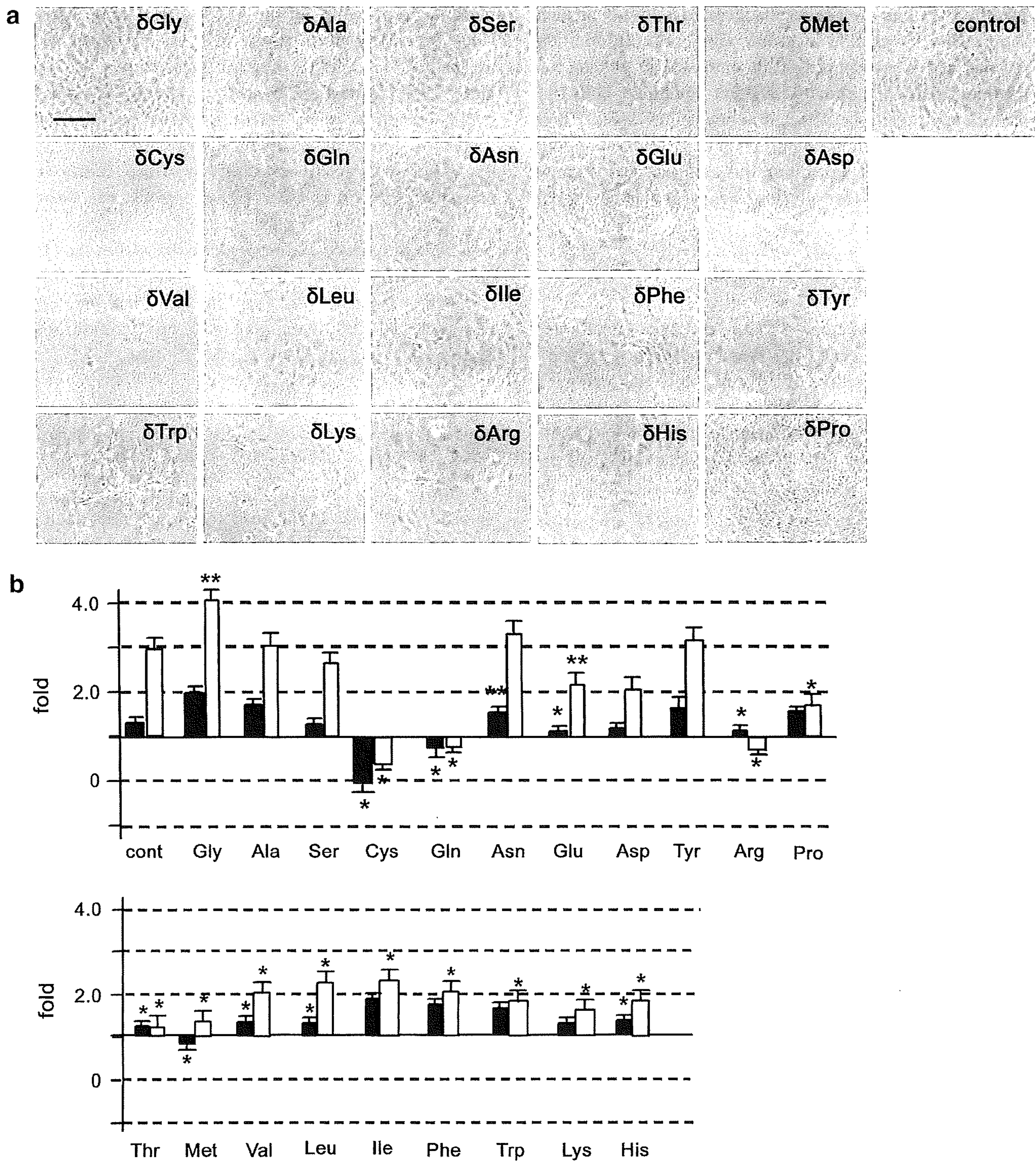


Fig. 2 Glycine depletion promotes cell proliferation of mSGP cells in short-term culture. **a** Morphology of mSGP cells at 48 h; this morphology was maintained in all cultures. *Bar* 100 μm. **b** Effects of amino acid depletion on mSGP cells proliferation (*y-axis* number of proliferating cells at each time point/number of cells at 0 h, *black bars* 24 h, *white bars* 48 h). Glycine-free medium demonstrated proliferation promotion compared with control (3.89±0.24-fold in δGly, 3.17±0.23-fold in control, $^{**}P<0.05$). Depletion of L-cysteine, L-glutamine, L-glutamic acid, L-aspartic acid, L-arginine, or L-proline resulted in a reduction in cell number at 48 h (0.73±0.05-fold in δCys, 0.87±0.04-fold in δGln, 2.29±0.21-fold in δGlu, 2.33±0.35-fold in δAsp, 0.97±0.06-fold in δArg, 1.84±0.09-fold in δPro, $^{*}P<0.001$, $^{**}P<0.05$). Depletion of L-threonine, L-methionine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-tryptophane, L-lysine, or L-histidine also resulted in a reduction in cell number (48 h: 1.09±0.11-fold in δThr, 1.39±0.19-fold in δMet, 2.11±0.12-fold in δVal, 2.32±0.20-fold in δLeu, 2.30±0.22-fold in δIle, 1.94±0.16-fold in δPhe, 2.27±0.39-fold in δTrp, 1.89±0.15-fold in δLys, 2.52±0.19-fold in δHis, $^{*}P<0.001$, $^{**}P<0.05$)

modified MTT assay. The results revealed a dose-dependent suppression by glycine in proliferating cells. In addition, the number of proliferating cells increased in glycine-free medium (data not shown). Neither apoptotic cells nor necrotic cells could be detected in any of the media. These results suggested that the suppression of cell proliferation in glycine-supplemented medium was not caused by amino acid toxicity, and that glycine was a regulator of cell proliferation.

Glycine induces cell-cycle arrest/delay in the S-phase

We investigated whether glycine influenced cell-cycle progression. mSGP cells were synchronized in the G0/G1-phases in the presence of glycine, and the cell-cycle distribution pattern was analyzed by quantifying the DNA content with flow cytometry analysis at 24 h after glycine treatment. Treatment with 10 mM glycine increased the percentage of cells in the S-phase (Fig. 3a), whereas the percentage of cells in the G2/M-phase was decreased (Fig. 3a). The percentage of cells in the G1-phase was unchanged (Fig. 3a). To confirm these results, we investigated cyclin expression by flow cytometry analysis. The cyclinA-positive cells, which appeared in the S-phase increased after 24 h glycine treatment, whereas the cyclinD1-positive or cyclinB1-positive cells, which appeared in the G1 or G2/M-phase, significantly decreased (Fig. 3b). These results suggested that glycine inhibited the cell-cycle progression in the S-phase, but not in the G0/G1-phase. These effects of glycine were independent of apoptosis (Supplemental Fig. 1a, b). Therefore, glycine regulated the cell cycle and cell proliferation without toxicity.

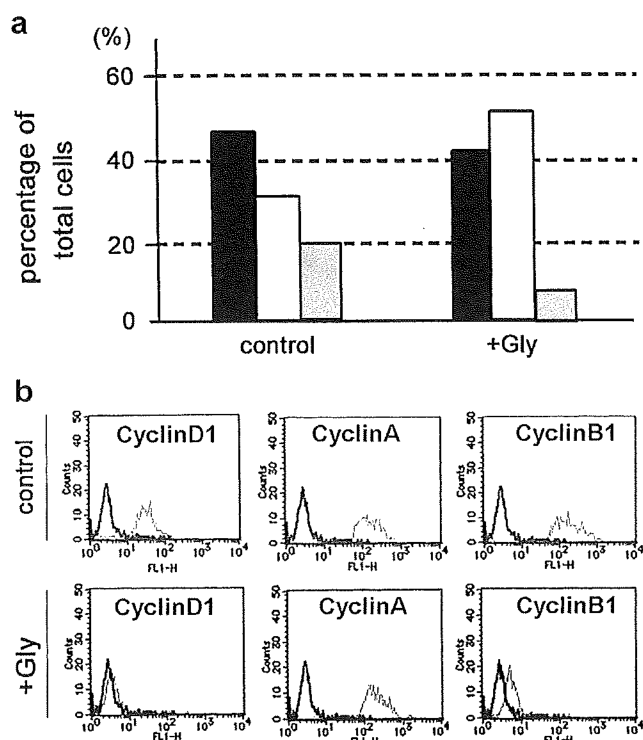
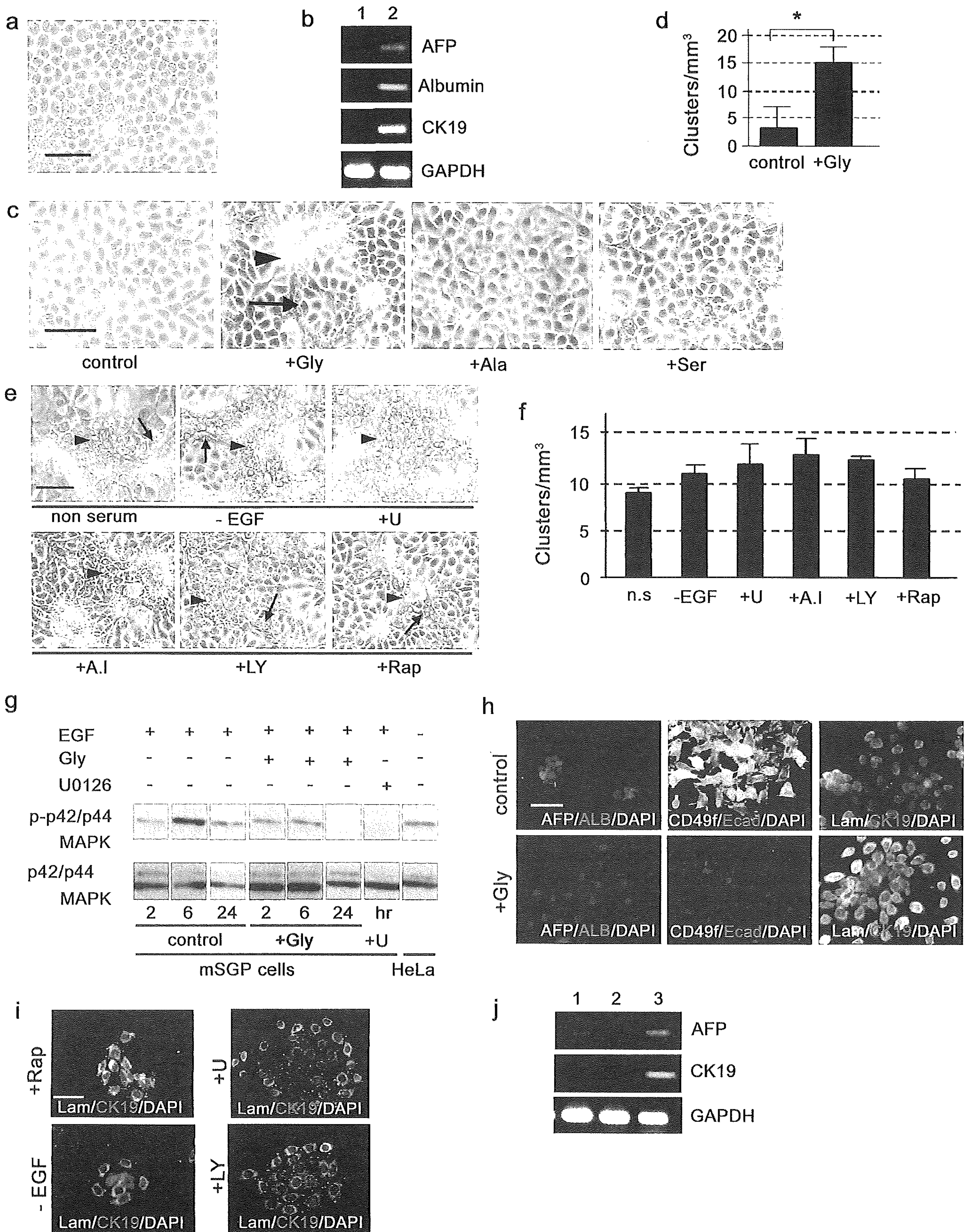


Fig. 3 Glycine affects on cell-cycle progression. Cell-cycle distribution was analyzed by quantification of DNA content and cyclin expression at 24 h after 10 mM glycine treatment. **a** Quantitative analysis of nuclear DNA contents by using Vybrant Dye-Cycle Green Staining. Glycine increased the percentage of cells in the S-phase compared with control (*control*: 45.95% [*black bar* G1-phase], 32.24% [*white bar* S-phase], 21.81% [*gray bar* G2/M-phase]; *+Gly*: 43.51% [*black bar* G1-phase], 49.75% [*white bar* S-phase], 6.75% [*gray bar* G2/M-phase]). **b** Flow cytometry analysis of cyclin expression. The numbers of cyclinA-positive cells increased after glycine treatment, whereas the numbers of cyclinD1-positive or cyclinB1-positive cells decreased (*gray lines*)

Fig. 4 Glycine promotes differentiation of mSGP cells in long-term culture. To investigate the effects of glycine on cell differentiation, mSGP cells were cultured for 2 weeks in medium supplemented with 10 mM glycine, and the cell morphology and gene expression were estimated by immunofluorescent stain and RT-PCR. **a** Morphology of immature mSGP cells. Cells formed uniform polygonal cell populations with large nuclei, assuming the form of an epithelium. *Bar* 100 μm. **b** RT-PCR performed on mRNA obtained from mSGP cells cultured in control medium; the cells expressed alpha-fetoprotein (*AFP*) but were negative for cytokeratin 19 (*CK19*) and albumin (*lane 1* mSGP cells in control medium, *lane 2* positive control, *GAPDH* D-glyceraldehyde-3-phosphate dehydrogenase). **c** Morphology of mSGP cells treated with glycine for 2 weeks. Small cell accumulation, cluster formations (*arrowhead*), and duct-like structures (*arrow*) were increased. The duct-like structures could not be detected in other media. *Bar* 100 μm. **d** Effect of glycine on cluster formation. Numbers of clusters of 1 mm^2 were counted. Clusters comprised differentiated mSGP cells. Glycine promoted the numbers of clusters compared with the control (*control*: 3.6±2.63 clusters/mm^2; *+Gly*: 14.7±4.17 clusters/mm^2, $*P<0.001$). **e** Morphology of mSGP cells in cultures with serum withdrawal (*non serum*), EGF withdrawal (*-EGF*), and signal transduction inhibitors (*+U* 5 μM U0126, *+LY* 20 μM LY294112, *+A.I* 20 μM Akt-inhibitor, *+Rap* 5 nM rapamycin). Cluster formations (*arrowheads*) and duct-like structures (*arrows*) were increased in all cultures. *Bar* 100 μm. **f** The effect of serum withdrawal (*n.s*), EGF withdrawal, and cell proliferation inhibitors on cluster formation. The number of clusters was not statistically different between the media. **g** Effect of glycine on EGF signaling pathway. Western blot analysis was performed on whole-cell lysates from cells cultured in 10 mM glycine or 5 μM U0126 supplementation for 2, 6, or 24 h. Glycine decreased phospho-p42/p44MAPK expression at 6 h in comparison with the control and abolished it at 24 h. Whole cell lysates from HeLa cells were used as a positive control (*+Gly* 10 mM glycine, *+U* 5 μM U0126, *HeLa* HeLa cells). **h, i** Immunofluorescent staining of mSGP cells. Immature mSGP cells cultured in control medium were positive for AFP, CD49f, and intracellular laminin (antigens of immaturity) and negative for CK19 (expressed by duct epithelium). **h** Cells cultured in glycine-containing medium for 2 weeks. Glycine decreased the expression of AFP (*green*), CD49f (*green*), and laminin (*green*), whereas it increased that of E-cadherin (*red*) and CK19 (*red*). *Bar* 100 μm. **i** Cells cultured with signal transduction inhibitors or without EGF. The expression of laminin (*green*) decreased and CK19 (*red*) increased in comparison with the control in **h**. *Bar* 100 μm. **j** RT-PCR performed on mRNA obtained from mSGPs cultured in glycine-containing medium. Glycine increased the gene expression of CK19 (*lane 1* control, *lane 2* +Gly, *lane 3* positive control)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Long-term glycine addition promotes differentiation of mSGP cells

Glycine has been suggested to promote the differentiation of mSGP cells. We investigated the effects of glycine on the differentiation of mSGP cells cultured for 2 weeks. The results showed a significant increase in differentiated clusters in glycine-containing medium (Fig. 4c, d). In addition, epithelial-duct-like structures appeared in mSGP cells cultured in this medium. The duct-like structures could not be detected in other media (Fig. 4c, arrow). The morphology of mSGP cells was unchanged with the other amino acids (Fig. 4c).

To investigate whether the effects of glycine on differentiation were associated with EGF signaling, we cultured mSGP cells in media supplemented with signal transduction inhibitors such as 5 μM MEK inhibitor (U0126), 20 μM PI3K inhibitor (LY294112), 20 μM Akt-inhibitor, or 5 nM rapamycin. The treatment with inhibitors led to an increase in clusters and duct-like structures, similar to the treatment with glycine (Fig. 4e, f). Western blotting analysis revealed that glycine decreased phospho-p42/p44MAPK and its activity at 6 h in comparison with control and completely abolished it at 24 h (Fig. 4g). Increased expression of CK19 was detected in cells cultured in glycine-supplemented medium, serum-free medium, EGF-free medium, or media with inhibitors (Fig. 4h-j). These results suggested that the effects of glycine on the differentiation of mSGP cells were similar to that of EGF-signal inhibition.

Discussion

In this study, we have demonstrated that glycine, which is one of the non-essential amino acids for mammals, suppresses the proliferation and promotes the differentiation of mSGP cells.

To investigate the effect of glycine on cell proliferation in vitro, we have cultured mSGP cells in the presence of glycine. Glycine supplementation suppresses cell proliferation of mSGP cells.

Our previous studies have demonstrated three procedures useful for investigating the differentiation of mSGP cells in three-dimensional culture; autonomous cell-cluster formation on monolayer culture, induced 3-D structure formation in matrigel matrix culture, and spherical culture (Hisatomi et al. 2004; Matsumoto et al. 2007; Okumura et al. 2003; Sato et al. 2007). The clear differences between each procedure are evident in terms of gene expression and function of mSGP cells. Cell clusters are formed autonomously on monolayer culture, and the differentiated mSGP cells in cell clusters are positive for CK19, whereas they are negative for insulin and albumin. This indicates that the cell-cluster formation on monolayer culture represents an early stage of mSGP cell differentiation. The cell culture procedures in matrigel and spherical culture also encourage mSGP cells to form 3-D structures, inducing differentiation. The differentiated cells in matrigel are of the hepatic lineage; they express albumin and antitrypsin and differentiate into hepatocytes after transplantation into the liver (Hisatomi et al. 2004; Okumura et al. 2003). Spherical cultures cause mSGP cells to differentiate into the pancreatic endocrine lineage via artificial 3-D structural formations. The differentiated cells in the spheres are positive for insulin and glucagon. These cells release insulin, when stimulated by glucose and potassium (Hisatomi et al. 2004; Matsumoto et al. 2007). In this study, we have used cell-cluster formation on monolayer culture to analyze the effect of glycine at the early stage of mSGP cell differentiation. Glycine supplementation on monolayer culture enhances cell-cluster formation of mSGP cells; the clusters are CK19 positive. Moreover, glycine supplementation encourages mSGP cells to form duct-like structures. The increase of autonomous cell-cluster formation by glycine suggests that glycine promotes mSGP cell differentiation. These results indicate that glycine exerts an effect on the differentiation of mSGP cells at an early stage.

We have also investigated the effect of signal transduction inhibitors, the removal of EGF, or glycine-supplementation on mSGP cells. Previous studies have shown that the phosphorylation and activation of members of the mitogen-activating protein kinase (MAPK) family such as p42/p44MAPK and p38MAPK, which are downstream effectors of the EGF receptor, occur during the development of several vertebral organs including the salivary glands (Cardoso and Lu 2006; Liu et al. 2008). Inhibition of PI3K, which is a downstream effector of the receptor of fibroblast growth factor (FGF), is reported to suppress the formation of the salivary gland epithelial bud, as shown when the MAPKs are inhibited (Larsen et al. 2003). The inhibitors and deprivation of EGF promote the formation of cell clusters, similar to the finding with glycine. EGF is an essential growth factor for mSGP cells to retain their immaturity. Glycine might therefore function to inhibit the EGF signaling pathway, leading to growth suppression, and to promote the differentiation of tissue progenitor cells.

During cell proliferation, growth factors are well known to affect cell-cycle progression, especially the G0 to G1 transition and the G1-S progression (Cardoso and Lu 2006; Jones and Kazlauskas 2001; Liu et al. 2008; Meloche and Pouyssegur 2007); hence, cell proliferation is suppressed by cell-cycle arrest in the G0/G1-phase (Jones and Kazlauskas 2001; Shackelford et al. 1999). When deprived of serum or growth factors, cells exit into

the G0-phase after they complete mitosis. These cells can be reintroduced into the cell cycle by the re-addition of serum or purified growth factors (Jones and Kazlauskas 2001; Pardee 1974, 1989). Growth factors such as EGF, FGF, and hepatocyte growth factor have recently been reported also to induce G2/M transition delay (Dangi et al. 2006; Nam et al. 2008). We have demonstrated that glycine induces S-phase arrest/delay in mSGP cells. The cell-cycle arrest at S-phase usually indicates a delay of DNA synthesis or DNA repair. A previous study has shown that the chemical inhibition of pyrimidine nucleotide synthesis in cells delays S-phase progression (Liu et al. 2007), indicating that S-phase arrest can indeed occur as a result of a delay in DNA synthesis. In this study, we have shown that glycine inhibits MAPK signaling (Fig. 4), suppresses cell proliferation (Fig. 1), promotes differentiation (Fig. 4), and arrests (or delays) the cell cycle (Fig. 3). Cells with DNA damage caused by radiation or chemical injury initiate cellular recovery mechanisms, such as cell-cycle arrest in the G1-phase and apoptosis (Delia et al. 1997; Gentile et al. 2003; Shackelford et al. 1999). Other reports have revealed that the deprivation of growth factors induces caspase and superoxide activities and cell apoptosis (Lieberthal et al. 1998). Our results provide no evidence of cell death resulting from apoptosis or necrosis after glycine supplementation. Furthermore, glycine-treated cells are induced to proliferate by the removal of glycine from culture media, suggesting that glycine has regulatory functions in cell proliferation, not in cell toxicity. The S-phase delay in non-proliferating cells treated with glycine might be caused by a delay in DNA synthesis or a delay in the G2/M transition accompanying the inhibition of growth factors.

Non-ketotic hyperglycenemia (NKH), which is an inherited deficiency of glycine metabolism, presents severe neurological symptoms and frequently accompanies brain malformations (Ichinohe et al. 2004; Sakata et al. 2001). It is characterized by the accumulation of a large amount of glycine in serum and cerebrospinal fluid (CSF), indicating that the normal glycine concentration in CSF is indispensable for normal brain development. In NKH, the oxidative breakdown of glycine is impaired, causing the deprivation of 5, 10-methylenetetrahydrofolate, which is essential for the synthesis of DNA during cell proliferation (Ichinohe et al. 2004; Sakata et al. 2001). This evidence suggests that a high concentration of glycine might have inhibitory or toxic effects on neurogenesis.

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Ⅳ. 先天性代謝異常症－2

遺伝性高チロシン血症

Hereditary tyrosinemia

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1 基本病因，発症機序

遺伝性高チロシン血症には種々の原因があり，3つの病型に分類されている(表)。これらの疾患は，遺伝的・酵素学的に別の疾患であり，臨床症状出現の機序も異なる[1]。遺伝形式はいずれも常染色体劣性である。

①遺伝性高チロシン血症Ｉ型[MIM 276700, hereditary tyrosinemia type Ｉ]：フマリルアセト酢酸ヒドラーゼ(FAH：EC 3.7.1.2)が欠損することで発症する(図)。

②遺伝性高チロシン血症Ⅱ型[MIM 276600, hereditary tyrosinemia typeⅡ]：細胞質チロシンアミノ基転移酵素(TAT：EC 2.6.1.5)の欠損症で，眼皮膚型高チロシン血症，Richner-Hanhart 症候群ともよばれる(図)。

③遺伝性高チロシン血症Ⅲ型[MIM 276710, hereditary tyrosinemia type Ⅲ]は 4-ヒドロキシフェニルピルビン酸酸化酵素(HPD：EC 1.13.11.27)が欠損している(図)。また，ホーキンシン尿症は HPD 異常により発症する常染色体優性遺伝性疾患である(表)。

2 基本病態

① Ｉ型では，酵素欠損によって細胞内に蓄積するフマリルアセト酢酸の毒性のために種々の病態が生じる[2〜5]。肝細胞では遺伝子発現の異常，酵素活性の阻害，アポトーシス，染色体の不安定および癌化が生じている。低血糖，アミノ酸やその他の代謝障害，凝固因子の低下などは，遺伝子発現の低下が原因であると考えられる。また，染色体の不安定性によって高い頻度で若年性肝臓癌が出現する。さらにアポトーシスによる肝細胞死によって肝不全が進行する。近位尿細管においても細胞障害が出現し，アミノ酸尿，糖尿，代謝性アシドーシスなどの Fanconi 症候群が発症する。その結果，低リン血症性くる病となる。

② Ⅱ型では，体液中の高いチロシン濃度によっ

表 高チロシン血症の分類

病型	遺伝性	血漿中のチロシン上昇	酵素欠損	主な症状
遺伝性高チロシン血症Ｉ型	常劣	軽度	フマリルアセト酢酸分解酵素	肝細胞障害，尿細管障害，低血糖，ガラクトース代謝異常，神経症状，肝細胞癌
遺伝性高チロシン血症Ⅱ型	常劣	高度	チロシンアミノ基転移酵素	精神発達遅延，皮膚の異常角化，角膜びらん，潰瘍
遺伝性高チロシン血症Ⅲ型	常劣	中等度	4ヒドロキシフェニルピルビン酸酸化酵素	失調，けいれん，軽度の精神発達遅延
ホーキンシン尿症	常優	一過性	4ヒドロキシフェニルピルビン酸酸化酵素	一過性発育遅延，食欲不振
肝障害に伴う高チロシン血症	原疾患による	さまざま		原疾患による
新生児一過性高チロシン血症	なし	さまざま		無症状または不活発

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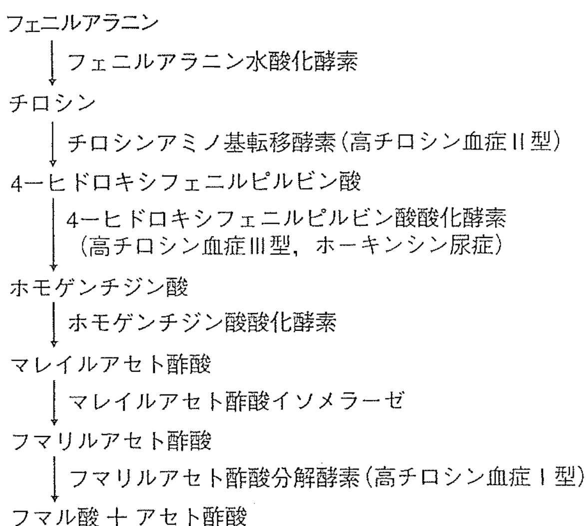

図　遺伝性高チロシン血症の代謝障害部位

て臨床症状を呈する。この症状の一部はチロシンの溶解度が低いことと関連している。皮膚や角膜ではほかの部位よりも温度が低下しやすく，チロシン結晶が析出しやすいことによって細胞障害を生じると考えられる。一方，精神発達の遅延も多くの患者で観察されている。これは血中チロシン値の上昇と関連して出現しているが，機序の詳細は不明である。また，患者尿中へはチロシンのアミノ基転移反応の生成物である4-ヒドロキシフェニルピルビン酸とその酸化物が大量に排泄されている。これは別の遺伝子にコードされたミトコンドリア局在性のチロシンアミノ基転移酵素により生成されたものである。

③ Ⅲ型では，チロシンのαケト酸である4-ヒドロキシフェニルピルビン酸とチロシンが増加する。尿中への4-ヒドロキシフェニルピルビン酸とその酸化物の排泄も著明に増加している。この疾患のマウスモデル（Ⅲマウス）では血中チロシン値は約20 mg/dl でⅢ型患者の値に近い[6]。

3 臨床症候

① Ⅰ型では，肝実質細胞と近位尿細管細胞の障害を認める。臨床的には，進行する肝障害と腎尿細管障害が特徴である。急性型，亜急性型，慢性型の3つの病型があり，急性型では生後数週から始まる肝腫大，発育不良，下痢，嘔吐，黄疸などがみられる。重症例では肝不全へ進行し，無治療であれば生後2～3か月で死亡する。亜急性型では，生後数か月～1年程度で肝障害を発症する。慢性型では肝障害の進行は緩やかであるが，最終的には肝硬変，肝不全にいたる。肝臓癌を発生する症例も多く，多発性腫瘍も報告されている。一方，腎臓では尿細管機能障害が出現し，低リン血症性くる病，ビタミンD抵抗性くる病などが認められる。また，サクシニルアセトンがアミノレブリン酸デヒドラターゼを阻害する結果，腹痛発作，ポリニューロパチーなどの急性間欠性ポルフィリン症に類似した症状が出現する。臨床症状の重症度と遺伝子型には関連を認める。

② Ⅱ型では，Ⅰ型やⅢ型より血中チロシン値が高く，Ⅰ型のような肝・腎障害を認めない。Ⅱ型の皮膚病変はチロシンの針状結晶が析出することによって出現し，手掌・足底に限局した過剰角化，びらんを生じる。また角膜においてもチロシンの結晶が析出し，角膜のびらん・潰瘍が生じる。角膜の変化は皮膚症状より早く出現し，生後数か月からみられるが，思春期以降に明らかになる症例もある。血中チロシン濃度がとくに高い一部の症例では精神発達の遅れを認めることがある。

③ Ⅲ型の症状はⅠ型，Ⅱ型よりも軽度であり，無症状の症例も存在する。これまでに失調，けいれん，軽度の精神発達遅延などが報告されている。これらはⅠ型，Ⅱ型にはみられない症状であり，体液中における4-ヒドロキシフェニルピルビン酸の増加が関連している可能性がある。このような症状をきっかけに診断される症例が少なくないことから，無症状の症例が診断されずに存在していると考えられる。

4 臨床検査

血中チロシンが高値である患者では高チロシン血症Ⅰ型，Ⅱ型，Ⅲ型以外に，ほかの原因による血中チロシン値の高値を鑑別する必要がある。Ⅰ型の鑑別診断では肝障害の原因となる疾患が重要である。またⅡ型，Ⅲ型の鑑別では，新生児一過性高チロシン血症が問題となる。血中チロシン値の経過観察には，血中アミノ酸分析やタンデム質量分析が有用である。

① 遺伝性高チロシン血症Ⅰ型の診断では肝障害

の有無が重要である。肝機能障害の結果，血清トランスアミナーゼの上昇や凝固因子の合成低下などを認める。腎尿細管機能障害により低リン酸血症，糖尿，蛋白尿などが認められる。また，血清中αフェトタンパクの増加が特徴的である。血中アミノ酸分析ではチロシンのほかメチオニン，セリン，スレオニンなどの多くのアミノ酸が上昇する。尿中アミノ酸分析ではチロシンをはじめとする多くのアミノ酸の排泄が増加している。ポルフィリン代謝障害の結果，尿中δアミノレブリン酸が増加する。画像診断では肝腫大，肝硬変や脂肪肝の所見がみられる。肝生検では肝構築の乱れや肝細胞の形態の異常，脂肪肝などを認める。しかしこれらの所見は非特異的であり，確定診断にはいたらない。

確定診断のためには，尿有機酸分析を行いチロシン代謝産物である4-ヒドロキシフェニルピルビン酸，4-ヒドロキシフェニル乳酸，4-ヒドロキシフェニルピルビン酢酸などの増加と，サクシニルアセトンの増加を明らかにする。尿中サクシニルアセトンの増加は診断的な価値が高い。また，酵素診断は肝細胞，培養皮膚線維芽細胞を検体として，フマリルアセト酢酸ヒドラーゼ活性を測定する。これらの尿中有機酸分析や酵素診断を行うことで診断を確定することができる。

②遺伝性高チロシン血症II型では皮膚や眼の所見から本症を疑われる。眼の症状はherpes simplex keratitisとの鑑別が問題となることがある。血中アミノ酸分析では血中チロシンは20 mg/dl以上ときわめて高値である。また，尿有機酸分析では4-ヒドロキシフェニルピルビン酸，4-ヒドロキシフェニル乳酸，4-ヒドロキシフェニルピルビン酢酸が大量に見いだされる。酵素活性の測定には肝生検が必要である。チロシンアミノ基転移酵素には，可溶画分に存在するsTATとミトコンドリアに局在するmTATがある。この疾患ではsTATだけが欠損しているため，酵素診断においてはこの両者を区別して測定する。

③遺伝性高チロシン血症III型では臨床症状は特徴的ではない。血中アミノ酸ではチロシンが約20 mg/dl程度まで増加し，尿中へ4-ヒドロキシフェニルピルビン酸およびその酸化物が大量に検出される。確定診断では肝酵素を測定する。III型の軽症型であるホーキンシン尿症は尿中ホーキンシンを検出することで診断される。

5 治療目標とその手順および症状検査からみた効果判定指標

チロシン高値の患者ではI型，II型，III型とその他の原因による高チロシン血症の鑑別を対症療法と同時に行う。新生児期には臓器障害がなければ基本的には経過観察する。

①I型では肝障害の進行を早期に防止することが重要であり，4-ヒドロキシフェニルピルビン酸酸化酵素の阻害剤(NTBC：2-(2-nitro-4-trifluoromethyl-benzoyl)-1,3-cyclo-hexanedione)を使用し，食事療法(低フェニルアラニン・低チロシン食)を併用する[7]。早期に治療を開始するとNTBCに反応する例が多いといわれている。治療の効果判定には肝機能検査と血清αフェトタンパク値の測定が有用である。血清αフェトタンパクを正常範囲に保つことができれば予後が期待できる。NTBCを使用しない例では肝不全にいたることが多く，肝移植が行われる。また，NTBCを使用した例でも肝臓癌の発生例では肝移植が行われる。NTBCは国内では入手困難であり，個人輸入が必要となる。

②II型では血液中のチロシン値を低下させることを治療の目標とする。チロシン値の低下に伴って皮膚および眼の症状は改善する。そのため，低フェニルアラニン・低チロシン食を行い，血中チロシン値を10 mg/dl以下に保つ。

③III型ではII型と同様に，低フェニルアラニン・低チロシン食による食事療法を行う。

6 合併症の病態生理とその診断治療予防

I型の合併症である肝臓癌と低リン血症性くる病が重要である。NTBCで早期治療を行った症例でも肝臓癌が発生することがある。そのため治療中は肝臓癌の早期発見に努める。

7 症状経過，予後判定

I型ではNTBC治療の効果を認めれば予後は期待できる。II型，III型での予後は比較的良好であ

る。

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概論：血中アミノ酸分析によって診断できる先天性アミノ酸代謝異常症

Inborn errors of amino acid metabolism

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Key words：アミノ酸，先天代謝異常症，アミノ酸分析，高アンモニア血症，タンデム質量分析

はじめに

臨床検査における血中アミノ酸分析は，2種類のカラムを用いた自動分析計によって血漿を試料として測定されている．約40種類のアミノ酸を同時に測定することにより短時間での解析が可能である．その原理は，液体クロマトグラフィを用いて酸性，中性，塩基性のアミノ酸を分画し，ニンヒドリン試薬と反応させ，各アミノ酸の溶出時間と吸光度を測定する．血液検体は，採血後直ちに血漿分離し−20℃保存すれば，シスチン以外のアミノ酸は約1カ月程度安定である．血漿分離後に4℃保存した検体は，数時間以内に測定することが望ましい．

先天性アミノ酸代謝異常症は，新生児マススクリーニングの対象である比較的頻度の高い疾患や，これまでわずかな報告しかない疾患など多彩である．そのほとんどはアミノ酸の代謝経路の遺伝的障害によって発症する．その結果，アミノ酸やその代謝物質の過剰な蓄積や欠損によって症状を呈する．確定診断のためには，それぞれの疾患に特徴的な症状と，臨床検査や酵素活性，遺伝学的検査などを駆使する必要がある．臨床検査としては，血糖，血液ガス，血中アンモニア，乳酸，ピルビン酸，血中アミノ酸分析，尿中アミノ酸分析，尿中有機酸分析などが有用なことがある．また，近年タンデム質量分析を用いたアミノ酸，アシルカルニチン分析が可能になっており，スクリーニング検査としての有用性が高まっている．

1. タンデム質量分析

タンデム質量分析法による新生児の先天代謝異常症スクリーニング(タンデムマススクリーニング)によって，これまで診断が困難であった有機酸代謝異常症，脂肪酸代謝異常症，アミノ酸代謝異常症の早期診断が可能になった．このスクリーニングは，米国の一部の州で始まり，米国各州，ヨーロッパ，アジアの一部の国々に普及している．我が国でも厚生労働省の班研究を中心としたパイロットスタディなど，一部の地域でこのスクリーニング検査が行われている．タンデム質量分析を用いることで，新生児期に20種類以上の疾患を発見できるとされている．その頻度は，我が国のパイロット研究では約8,000人に1人と推定される．これらの疾患を発見することによって，先天代謝異常症による突然死や精神運動発達遅滞を予防できる可能性がある．

2. 新生児マススクリーニング

アミノ酸代謝異常症のマススクリーニングは，

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我が国では30年以上の歴史を有し，数多くの患者の早期診断・早期治療が達成されている．新生児マススクリーニングの対象になっている先天性アミノ酸代謝異常症はフェニルケトン尿症，ホモシスチン尿症，メープルシロップ尿症の3疾患である．我が国では生後3–5日目に，ろ紙血を採取し血中のフェニルアラニン，メチオニン，ロイシンを測定し，それぞれフェニルケトン尿症，ホモシスチン尿症とメープルシロップ尿症を診断する．古典的な方法としてGuthrie法がある．この方法は特殊なアミノ酸要求変異枯草菌を利用する一種のバイオアッセイ法で，現在でも一部の地域では使用されている．最近では酵素法や液体クロマトグラフィ法も利用されている．欧米ではタンデムマスを利用した簡易測定法が新生児マススクリーニングなどに応用されつつあり，我が国においてもその導入が準備されている．スクリーニングにおけるカットオフ値はフェニルアラニン4mg/dL以上，メチオニン2mg/dL以上，ロイシン4mg/dL以上である．

3. 血中アミノ酸の異常と疾患(表1)

a. フェニルアラニンの代謝障害

血中のフェニルアラニンが増加する疾患には，フェニルアラニン水酸化酵素異常症と，この酵素の補酵素であるテトラヒドロビオプテリン(BH_4)欠損症がある．これらは新生児マススクリーニングの対象疾患となっている．前者は遺伝子変異によって代謝障害の重症度にばらつきがあり，重症例は古典型フェニルケトン尿症と呼ばれる．古典型フェニルケトン尿症では血中フェニルアラニンが20mg/dLを超える状態が持続すると，精神運動発達の遅れが出現する．また，10mg/dL以上でもその危険がある．一方，食事療法を必要としない軽症高フェニルアラニン血症も知られている．フェニルアラニン水酸化酵素異常症とBH_4欠損症との鑑別は，BH_4負荷試験および尿中ビオプテリン代謝産物の測定で行う．

b. 遺伝性高チロシン血症

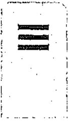
遺伝性高チロシン血症には遺伝的に異なった3つの病型がある．I型はフマリルアセト酢酸分解酵素，II型はチロシンアミノ基転移酵素，III型は4–ヒドロキシフェニルピルビン酸酸化酵素の欠損による．I型では肝障害と腎尿細管障害が特徴で，重症例では乳児期早期から肝障害が進行する．軽症例でも1–3歳で肝移植が必要になる．II型はRichner–Hanhart症候群とも呼ばれ，軽度の精神発達の遅れ，皮膚の過剰角化とびらん，角膜の異常などがみられる．III型では軽度の精神発達の遅れ，けいれん，失調などが報告されている．血中アミノ酸分析ではI型ではチロシン高値のほか，メチオニン高値がよくみられ，肝障害，肝硬変を合併している．また尿の有機酸分析では尿中へのサクシニルアセトンの排泄がI型の特徴である．II型，III型ではチロシンのみの高値がみられる．尿の有機酸分析では，すべての病型でチロシンの代謝産物の排泄が増加している．

c. メープルシロップ尿症

分岐鎖ケト酸の代謝障害があり，血中アミノ酸分析では分岐鎖ケト酸であるロイシン，イソロイシン，バリンが増加している．患者では分岐鎖ケト酸脱水素酵素複合体の活性が著明に減少しており，この酵素の構成成分であるE1α，E1β，E2，E3遺伝子の異常によって発症する．E3は他の酵素複合体にも作用しており，やや異なる臨床像を呈する．本症の臨床病型は，新生児期に発症する古典型，これよりも遅れて発症する間欠型や中間型，ビタミンB_1反応型などに分類されている．これらの病型は血中分岐鎖アミノ酸の上昇の程度と関連している．最も重症の古典型では生後すぐから哺乳力低下，嘔吐で発症し意識障害，呼吸障害，けいれんへと進行する．間欠型は新生児期には特に症状がなく経過し，その後古典型と同様の症状が発作性に出現する．これらの症状は分岐鎖アミノ酸のアミノ基転移反応の結果生じるケト酸の蓄積に伴うものである．治療効果の判定には血中ロイシンを測定し評価する．血中ロイシン値の高値(約13mg/dL以上)が持続すると知的予後は不良である．新生児マススクリーニングの対象疾患であり，ロイシン値を測定することにより診

表1 主な先天性アミノ酸代謝異常症(高アンモニア血症を除く)

	遺伝的代謝障害部位	主な臨床症状	臨床検査における血中アミノ酸異常
古典型フェニルケトン尿症	フェニルアラニン水酸化酵素	精神運動発達遅延 皮膚・毛髪の色素低下	フェニルアラニンの上昇
高フェニルアラニン血症	フェニルアラニン水酸化酵素	皮膚・毛髪の色素低下	フェニルアラニンの上昇
テトラヒドロビオプテリン欠損症	様々なビオプテリン代謝障害	重度の精神発達遅延	フェニルアラニンの上昇
高チロシン血症			
I型	フマリルアセト酢酸分解酵素	進行する肝腎障害，肝癌	チロシン，メチオニンその他の上昇
II型	チロシンアミノ基転移酵素	精神発達遅延，皮膚・眼の異常	チロシンの上昇
III型	4-ヒドロキシフェニルピルビン酸酸化酵素	精神発達遅延，けいれん，失調	チロシンの上昇
ホーキンシン尿症	4-ヒドロキシフェニルピルビン酸酸化酵素	一過性の哺乳力低下，発育遅延	チロシンの上昇
高ヒスチジン血症	ヒスチダーゼ	特に症状はない	ヒスチジンの上昇
メープルシロップ尿症			
E1α欠損 E1β欠損 E2欠損	分岐鎖ケト酸脱水素酵素複合体	けいれん，呼吸障害 意識障害，精神運動発達遅延	ロイシン，イソロイシン，バリンの上昇
ホモシスチン尿症	シスタチオニンβ合成酵素	精神運動発達遅延，マルファン様手足，水晶体脱臼	メチオニン，ホモシスチンの上昇
高グリシン血症	グリシン開裂酵素複合体	重度の精神運動発達遅延	グリシンの上昇
高プロリン血症			
I型	プロリン酸化酵素	けいれん，精神発達の遅れ？	プロリンの上昇
II型	ピロリン5カルボン酸脱水素酵素	けいれん，精神発達の遅れ？	プロリンの上昇
ヒドロキシプロリン血症	ヒドロキシプロリン水酸化酵素	特に症状はない	ヒドロキシプロリンの上昇
高リジン血症	アミノアジピン酸セミアルデヒド合成酵素	特に症状のない症例が多い	リジンの増加
高アラニン血症	ピルビン酸脱水素酵素複合体 ミトコンドリア障害など	高乳酸血症の原因に関連した症状，精神運動発達遅延，けいれん，呼吸障害，筋緊張低下	アラニン，プロリンの上昇
Hartnup病	中性アミノ酸の吸収障害	トリプトファン欠乏に伴うペラグラ	トリプトファンおよび中性アミノ酸の低下



D 低分子窒素化合物関係

表2　高アンモニア血症をきたすアミノ酸代謝異常症

	高アンモニア血症以外の特徴的な所見	代謝障害部位	アミノ酸の異常
アセチルグルタミン酸合成酵素欠損症	オロト酸排泄増加なし	アセチルグルタミン酸合成酵素	アラニン，グルタミン，グルタミン酸の高値
カルバミルリン酸合成酵素欠損症	オロト酸排泄増加なし	カルバミルリン酸合成酵素	シトルリン，アルギニンの低値，時にオルニチンの高値
オルニチントランスカルバミラーゼ欠損症	オロト酸排泄増加あり	オルニチントランスカルバミラーゼ	グルタミン，グルタミン酸の高値，シトルリンの低値
シトルリン血症(古典型)		アルギニノコハク酸合成酵素	シトルリンの高値
アルギニノコハク酸尿症	肝脾腫	アルギニノコハク酸分解酵素	アルギニノコハク酸の上昇
アルギニン血症	痙性対麻痺	アルギナーゼ	アルギニンの上昇
HHH症候群*		オルニチン膜転送	オルニチンの上昇
高オルニチン血症**	脈絡膜脳回転状変性	オルニチンアミノ基転移酵素	オルニチンの上昇
リジン尿症	肝脾腫，骨粗鬆症	リジン膜転送	リジン，アルギニン，オルニチンの低値
シトリン異常症	肝障害	シトリンの欠損	シトルリンのほか種々のアミノ酸の増加

*HHH症候群：高オルニチン血症・高アンモニア血症・ホモシトルリン血症症候群.
**高オルニチン血症：新生児期はオルニチンは低値で，高アンモニア血症が生じる.

断される.

d. ホモシスチン尿症と高メチオニン血症

新生児マススクリーニングの対象疾患であり，血中メチオニンが増加している．シスタチオニンβ合成酵素の欠損によりホモシステインの代謝が障害され，ホモシステインはホモシスチンへ酸化され尿中へ排泄される．未治療の患者では，精神発達の遅れ，眼症状(水晶体脱臼)，マルファン様手足や血栓症などがみられる．メチオニンの高値を示す遺伝性代謝性疾患の種類は多く，鑑別には注意を要する．また重症の肝障害でも高値を呈する．特に新生児期には一過性の高メチオニン血症を呈する例や，その他の酵素異常による高メチオニン血症を鑑別する必要がある.

e. グリシンとプロリンの異常

高グリシン血症はグリシン開裂反応の遺伝的異常(非ケトーシス型高グリシン血症)によって生じる．この疾患では，重度の神経症状が出現しても血中グリシン値の上昇は軽度のことがあるため，本症の疑いがあれば髄液のアミノ酸分析でグリシンの高値を検出する.

プロリンの高値は，遺伝性高プロリン血症I型とII型でみられる．臨床症状との関連はよくわかっていない．遺伝性高プロリン血症では血中プロリンの値は正常上限の5-10倍に上昇する．ヒドロキシプロリン血症は極めてまれである.

f. 高アンモニア血症(表2)

1) オルニチントランスカルバミラーゼ(OTC)欠損症とカルバミルリン酸合成酵素(CPSI)欠損症

血中アミノ酸分析ではグルタミン，グルタミン酸の高値，シトルリンの低値，時にオルニチンの高値がみられる.

2) シトルリン血症

アルギニノコハク酸合成酵素の欠損による．肝障害はないか，あっても軽度でありアミノ酸分析では血中シトルリンが著明に増加しアルギニンが低値である．尿中へのシトルリンも著明に増加している．重症例は新生児期に著明な高アンモニア血症で発症し，軽症例では乳児期以