

论著

原位杂交研究GPRC6A mRNA在小鼠组织中的分布规律

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摘要:

目的: 探讨G蛋白偶联受体C组6A (G protein-coupled receptor family C, group 6, subtype A, GPRC6A) mRNA在小鼠体内的分布规律。方法: 比较传统和TSA信号放大系统分析石蜡包埋的小鼠组织中GPRC6A mRNA的分布情况。为了确保信号特异性, 将GPRC6A基因剔除小鼠组织放在同一张切片上作阴性对照。结果: 建立了一种用非同位素标记的互补RNA探针原位杂交检测石蜡包埋的小鼠组织中低表达GPRC6A mRNA的方法。TSA信号放大系统明显提高原位杂交检测该受体的敏感性。GPRC6A基因剔除小鼠组织中没有可测信号。GPRC6A mRNA至少在消化腺、副消化腺中表达, 同时也在肾、睾丸、子宫、脑、肌肉脂肪中有表达。结论: GPRC6A mRNA的分布模式与GPRC6A基因剔除小鼠的基因表型基本一致。

关键词: GPRC6A mRNA 小鼠 分布模式 TSA信号放大系统 原位杂交

Distribution pattern of GPRC6A mRNA in mouse tissue by in situ hybridization

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Abstract:

ObjectiveTo explore the distribution pattern of G protein-coupled receptor family C, group 6, subtype A (GPRC6A) mRNA in adult mice. MethodsThe distribution of GPRC6A mRNA in paraffin embedded adult mouse tissues was determined by highly sensitive nonradioactive cRNA probe in situ hybridization (ISH). We compared ISH with and without addition of tyramide signal amplification (TSA). GPRC6A wild-type and littermate GPRC6A null mice tissue sections were investigated by ISH. ResultsTSA greatly increased the sensitivity of ISH to detect GPRC6A mRNA in wild type mouse tissues. There was no detection of GPRC6A mRNA in GPRC6A gene specific knockout tissue in paraffin embedded tissue section. The mRNA of GPRC6A was detectable in the digestive gland or accessory digestive gland including salivary gland and pancreas, as well as in the tissues including kidney, testis, brain, muscle, and fat. ConclusionThe mRNA distribution pattern of GPRC6A gene is compatible with the phenotype of GPRC6A knockout mice.

Keywords: GPRC6A; mRNA; mouse; distribution pattern; tyramide signal amplification; in situ hybridization

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- [1] Wellendorph P, Johansen L D, Brauner-Osborne H. Molecular pharmacology of promiscuous 7TM receptors sensing organic nutrients [J] . *Mol Pharmacol*, 2009, 76(3): 453-465.
- [2] Kuang D, Yao Y, Lam J, et al. Cloning and characterization of a family C orphan G-protein coupled receptor [J] . *J Neurochem*, 2005, 93(2): 383-391.
- [3] Wellendorph P, Brauner-Osborne H. Molecular cloning, expression, and sequence analysis of GPRC6A, a novel family C G-protein-coupled receptor [J] . *Gene*, 2004, 335, 37-46.
- [4] Pi M, Faber P, Ekema G, et al. Identification of a novel extracellular cation-sensing G-protein-coupled receptor [J] . *J Biol Chem*, 2005, 280(48): 40201-40209.
- [5] Brown E M, Gamba G, Riccardi D, et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid [J] . *Nature*, 1993, 366(6455): 575-580.
- [6] Pin J P, Duvoisin R. The metabotropic glutamate receptors: structure and functions [J] . *Neuropharmacol*, 1995, 34(1): 1-26.
- [7] Mohler H, Frisetschy J M. GABAB receptors makes it to the top—as dimmers [J] . *Trends Pharmacol Sci*, 1999, 20(3): 87-89.
- [8] Nelson G, Hoon M A, Chandrashekar J, et al. Mammalian sweet taste receptors [J] . *Cell*, 2001, 106(3): 381-390.
- [9] Nelson G, Chandrashekar J, Hoon M A, et al. An amino-acid taste receptor [J] . *Nature*, 2002, 416(6877): 199-202.
- [10] Robbins M J, Michalovich D, Hill J, et al. Molecular cloning and characterization of two novel retinoic acid-inducible orphan G-protein-coupled receptors (GPRC5B and GPRC5C) [J] . *Genomics*, 2000, 67(1): 8-18.
- [11] Brauner-Osborne H, Krogsgaard-Larsen P. Sequence and expression pattern of a novel human orphan G-protein-coupled receptor, GPRC5B, a family C receptor with a short amino-terminal domain [J] . *Genomics*, 2000, 65(2): 121-128.
- [12] Calver A R, Michalovich D, Testa T T, et al. Molecular cloning and characterisation of a novel GABAB-related G-protein coupled receptor [J] . *Brain Res Mol Brain Res*, 2003, 110(2): 305-317.
- [13] Conigrave A D, Quinn S J, Brown E M. L-amino acid sensing by the extracellular Ca<sup>2+</sup>-sensing receptor [J] . *Proc Natl Acad Sci USA*, 2000, 97(9): 4814-4819.
- [14] Folkers K, Bowers C Y, Tang P F, et al. Decapeptides as effective agonists from L-amino acids biologically equivalent to the luteinizing hormone-releasing hormone [J] . *Proc Natl Acad Sci USA*, 1986, 83(4): 1070-1074.
- [15] Christiansen B, Hansen K B, Wellendorph P, et al. Pharmacological characterization of mouse GPRC6A, an L-alpha-amino-acid receptor modulated by divalent cations [J] . *Br J Pharmacol*, 2007, 150(6): 798-807.
- [16] Wellendorph P, Hansen K B, Balsgaard A, et al. Deorphanization of GPRC6A: a promiscuous L-alpha-amino acid receptor with preference for basic amino acids [J] . *Mol Pharmacol*, 2005, 67(3): 589-597.
- [17] Wellendorph P, Burhenne N, Christiansen B, et al. The rat GPRC6A: Cloning and characterization [J] . *Gene*, 2007, 396(2): 257-267.
- [18] Pi M, Chen L, Huang M Z, et al. GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome [J] . *PLoS One*, 2008, 3(12): e3858.
- [19] Wellendorph P, Johansen L, Jensen A, et al. No evidence for a bone phenotype in GPRC6A knockout mice under normal physiological conditions [J] . *J Mol Endocrinol*, 2009, 42(3): 215-233.
- [20] Yang H, Wanner I B, Roper S D, et al. An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs [J] . *J Histochem Cytochem*, 1999, 47(4): 431-445.
- [21] Mitsuma T, Rhue N, Kayama M, et al. Distribution of calcium sensing receptor in rats: an immunohistochemical study [J] . *Endocrin Regul*, 1999, 33(3): 55-59.
- [22] Qian X, Lloyd R V. Recent developments in signal amplification methods for in situ hybridization [J] . *Diagn Mol Pathol*, 2003, 12(1): 1-13.
- [23] Qian X, Bauer R A, Xu H S, et al. In situ hybridization detection of calcitonin mRNA in routinely fixed paraffin-embedded tissue sections: a comparison of different type of probes combined with tyramide signal amplification [J] . *Appl Immunohistochem Mol Morphol*, 2001, 9(1): 61-69.
- [24] Feng J Q, Ward L M, Liu S, et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism [J] . *Nat Genet*, 2006, 28(11): 1310-1315.
- [25] Plummer T B, Sperry A C, Xu H S, et al. In situ hybridization detection of low copy nucleic acid sequences using catalyzed reporter deposition and its usefulness in clinical human papillomavirus typing [J] . *Diagn Mol Pathol*, 1998, 7(2): 76-84.
- [26] Singer R H, Lawrence J B, Villnave C. Optimization of in situ hybridization using isotopic and non-isotopic detection methods [J] . *Bio Techniques*, 1986, 4: 230-250.
- [27] Tabata T, Araishi K, Hashimoto K, et al. Ca<sup>2+</sup> activity at GABAB receptors constitutively promotes metabotropic glutamate signaling in the absence of GABA [J] . *Proc Natl Acad Sci USA*, 2004, 101(48): 16952-16957.
- [28] Tfelt-Hansen J, Brown E M. The calcium-sensing receptor in normal physiology and pathophysiology: a review [J] . *Crit Rev Clin Lab Sci*, 2005, 42(1): 35-70.

