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• *BRIEF REPORTS* •

Variations of very low-density lipoprotein receptor subtype expression in gastrointestinal adenocarcinoma cells with various differentiations

Tao Chen, Fan Wu, Feng-Ming Chen, Jun Tian, Shen Qu

Tao Chen, Fan Wu, Jun Tian, Shen Qu, Department of Biochemistry & Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Feng-Ming Chen, Department of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China
Supported by the National Natural Science Foundation of China, No. 30470872
Correspondence to: Professor Shen Qu, Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. qushen@mails.tjmu.edu.cn
Telephone: +86-27-83692623 Fax: +86-27-83692608
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Abstract

AIM: This study is aimed at investigating the expression and possible significances of very low-density lipoprotein receptor (VLDLR) subtypes in gastroenteric adenocarcinoma tissues and cells with various differentiations.

METHODS: Thirty-one cases of gastroenteric carcinoma/adjacent normal tissues were enrolled in the study, which were diagnosed and classified by the clinicopathological diagnosis. The expression of VLDLR subtypes was detected in gastroenteric carcinoma/adjacent normal tissues and three various differentiated human gastric adenocarcinoma cell lines (MKN28, SGC7901 and MKN45) by reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis.

RESULTS: Two VLDLR subtypes, namely, type II VLDLR and type I VLDLR, were found to express changes in gastroenteric carcinoma tissues, their adjacent normal tissue, and gastric adenocarcinoma cell lines as well. Type II VLDLR is predominantly expressed in poorly- or moderately-differentiated gastroenteric carcinoma tissues and gastric adenocarcinoma cell lines, whereas type I VLDLR is mainly detected in well-differentiated intestinal carcinoma tissues and gastric adenocarcinoma cells compared with the adjacent normal tissues.

CONCLUSION: The results suggested that the variations of the VLDLR subtype expression might be correlated with the progress and differentiation of gastroenteric carcinoma.

Key words: VLDLR; Gastric adenocarcinoma

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INTRODUCTION

Very low density lipoprotein receptor (VLDLR) is a member of the low-density lipoprotein receptor (LDLR) superfamily. VLDLR closely resembles LDLR in structure, but differs from LDLR in function[1,2]. According to the presence of *O*-linked sugar region located outside the VLDLR membrane, VLDLR is divided into two subtypes: full-length VLDLR (type I) and *O*-linked sugar region lacked VLDLR (type II)[3]. The studies about the distribution of these two VLDLR subtypes suggested that their distribution presents obvious tissue- and cell-specificity. For example, type I VLDLR is mainly distributed in tissues of heart and skeletal muscles with high lipid metabolism, while type II VLDLR predominates in the non-muscle tissues, such as kidney, spleen, adrenal gland, lung, brain, testis, uterus and ovary[4,5]. A recent study showed that VLDLR subtypes are relevant with the differentiation and development of tissues and cells. For instance, during embryonic development in mice, mRNA level of VLDLR is significantly fluctuated in the heart, kidney and brain tissues; however, it is relatively constant in skeletal muscles[6]. The mice with VLDLR deficiency only demonstrate slower development[7]. In addition, type II VLDLR has been found to be the major expressed receptor in embryonic tissues and germ cells in chicken, necessary for reproduction[8,9]. Other reports indicated that some cancer tissues and cells also express two VLDLR subtypes that possess inhomogeneity[10-12].

Abnormal differentiation of cells leads to cancer formation. However, the reports so far are hardly ever concerned about the identification of VLDLR subtype expression in various differentiated carcinoma tissues and cells. Therefore, we sought to determine whether the expression of VLDLR subtypes could show a degree of difference in gastroenteric carcinoma tissues, their adjacent normal tissue, and three various differentiated human gastric adenocarcinoma cell lines (MKN28, SGC7901 and MKN45) as well, in order to understand the possible

implication of VLDLR subtype expression during the occurrence, differentiation and progress of cancer.

MATERIALS AND METHODS

Tissues and cells

Thirty-one cases of gastroenteric carcinoma/adjacent normal tissue specimens were collected during 2001-2002 from patients in Tongji hospital (Wuhan, PR China), and were diagnosed and classified by the clinicopathological diagnosis. Of these, 10 cases of gastric carcinomas were poorly- or moderately-differentiated adenocarcinomas, and 21 cases were large intestine carcinomas, of which there were 6 cases of well-differentiated adenocarcinomas and 15 cases of poorly- or moderately-differentiated adenocarcinomas. The collected specimens were immediately placed in RNA TRIzol and stored in liquid nitrogen. Well-, moderately- and poorly-differentiated human gastric adenocarcinoma cell lines (MKN28, SGC7901 and MKN45) were donated from the department of gastroenterology, Union Hospital. Anti-VLDLR polyclonal antibodies for Western blot analysis were prepared at our laboratory[13].

Human gastric adenocarcinoma cell culture

Three of the variedly differentiated human gastric adenocarcinoma cell lines (MKN28, SGC7901 and MKN45) were cultured in RPMI1640 supplemented with 10% FBS, 0.22 sodium bicarbonate and 0.25% HEPES at 37 ℃ and 50 mL/L CO_2. After 48 h, all cultured cells were digested with 0.25% pancreatin, and harvested for RT-PCR and Western blot.

RT-PCR analysis

Total RNA was isolated from 2 g tissue specimens or 5×10^8 cells in reference to the manual of RNA Trizol. Four micrograms total RNA was reverse-transcripted to cDNA for PCR experiment. The sequences of PCR primers referring to Nakamura[14] were 2162F: CAACCTGAA-TGATGCCCAAGA, and 2496R: CTTTTGGGGGAA-CACTGACCT, which just span the *O*-linked sugar region of human VLDLR, thereby distinguishing two VLDLR subtypes. Cycling conditions were: denaturing at 94 ℃ for 4 min, then at 94 ℃ for 1 min, at 56 ℃ for 1 min, at 72 ℃ for 2 min for 35 cycles. Finally the mixture was extended at 72 ℃ for 10 min. The PCR products were electrophorized on 2% agarose gels, stained by ethidium bromide, photographed under ultraviolet lamp, and then analyzed with GIS-2000 scanning and analyzing system.

Western blot analysis

For Western blot, the cultures were rinsed twice in ice-cold PBS, lysed in buffer containing 50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, 0.02% sodium azide, 100 μg/mL phenylmethysulfonyl fluoride, 1 μg/mL aprotinin, followed by spinning at 13 000 *g* at 4 ℃ for 20 min. The supernatants were removed and denatured immediately by adding the same volume of 2× laemmli buffer. The protein concentration was determined with Lowry Protein Assay Reagent. The equal amounts of protein (80 μg) were isolated in 7.5% SDS-polyacrylamide gel, and blotted onto polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membranes were blocked with 5% BSA in TBS for 2 h at room temperature, then immunostained with the polyclonal antibodies against human VLDLR. The Western blots were developed by using enhanced chemiluminescence detection reagents, as described by the manufacturer.

Statistical analysis

The apex area ratio of VLDLR types II/I in all specimens was analyzed by *t* test with Microsoft Excel 2002. A *P* value less than 0.05 was considered statistically significant.

RESULTS

mRNA level of VLDLR subtypes in intestinal carcinomas compared to the adjacent tissues

Type I VLDLR (335 bp) and type II VLDLR (252 bp) were detected in both intestinal carcinoma and adjacent tissues. In comparison of carcinoma with normal tissues, however, expression of VLDLR subtypes showed obvious difference. Two hundred and fifty-two base pair bands were predominantly expressed in poorly- or moderately-differentiated intestinal carcinoma, whereas well-differentiated intestinal carcinoma mainly presented 335 bp bands (Figure 1). Statistical analysis also showed a significant difference of $P<0.05$ (Table 1).

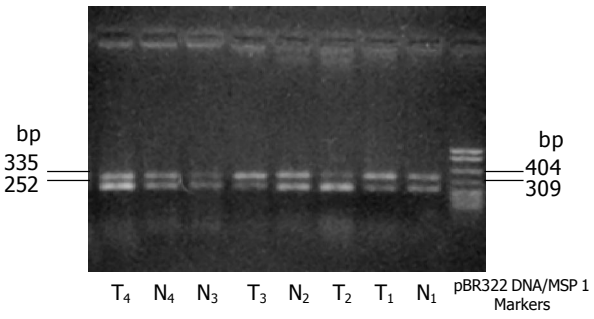

Figure 1 Detection of mRNA of VLDLR subtypes: type I (335 bp) and type II (252 bp) in intestinal carcinoma vs. adjacent tissues, T_1 and T_3: well-differentiated intestinal cancer, T_2 and T_4: moderately- or poorly-differentiated intestinal cancer, N_1, N_2, N_3 and N_4: adjacent tissues.

Table 1 VLDLR subtype expression ratio in gastroenteric cancer

	The apex area ratio of VLDLR type II/I	
	Tumor tissue	Adjacent tissue
Intestinal cancer		
Well-differentiated ($n=6$)	0.518±0.021[a]	0.834±0.011
Moderately- or poorly-differentiated ($n=15$)	2.091±0.495[c]	1.072±0.107
Gastric cancer		
Moderately- or poorly-differentiated ($n=10$)	2.214±0.215[e]	1.403±0.189

[a]$P<0.05$; [c]$P<0.05$; [e]$P<0.05$ *vs* adjacent tissue.

mRNA level of VLDLR subtypes in gastric carcinomas compared to the adjacent tissue

Both type I VLDLR (335 bp) and type II VLDLR (252 bp) appeared in both gastric carcinoma and the adjacent tissues. However, compared to normal tissues, the mRNA level of type II VLDLR in poorly- and moderately-differentiated gastric carcinoma were significantly higher than type I VLDLR, with a significant difference of $P<0.05$ (Figure 2, Table 1). Additionally, the collected gastric carcinoma specimens with lower expression of type I VLDLR were from the patients who had highly malignant degree of gastric carcinoma. These results implied that the expression of VLDLR subtypes might be related to the malignancy of gastric carcinoma.

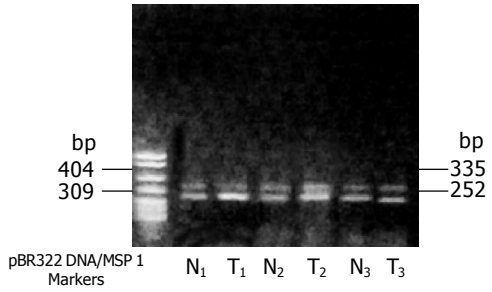

Figure 2 Detection of mRNA of VLDLR subtypes: type I (335 bp) and type II (252 bp) in gastric carcinoma *vs* adjacent tissues, T_1, T_2 and T_3: moderately- or poorly-differentiated gastric cancer, N_1, N_2 and N_3: adjacent tissues.

mRNA level of VLDLR subtypes in three various differentiated gastric adenocarcinoma cell lines

Both type I VLDLR (335 bp) and type II VLDLR (252 bp) presented in three various differentiated gastric adenocarcinoma cell lines (MKN28, SGC7901 and MKN45). However, poorly- or moderately-differentiated SGC7901 and MKN45 mainly expressed type II VLDLR, while well-differentiated MKN28 showed significantly higher level of type I VLDLR (Figure 3). These results coincided with that of gastric carcinoma tissues.

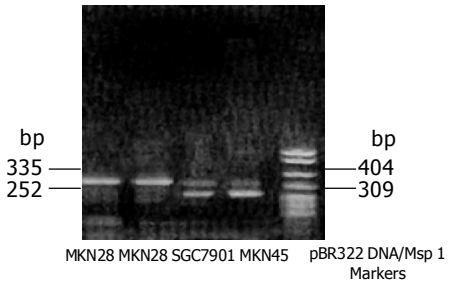

Figure 3 Detection of mRNA of VLDLR subtypes (335 and 252 bp) in MKN45, SGC7901 and MKN28 cell lines.

Protein level of VLDLR subtypes in various differentiated gastric adenocarcinoma cell lines

Protein of VLDLR subtypes was detected by Western blot. The results showed the protein expression of both type I VLDLR (155 ku) and type II VLDLR (130 ku) in well-differentiated MKN28, but the 155 ku band was the major one. Significantly, in poorly- or moderately-differentiated SGC7901 and MKN45 cell lines, only type II VLDLR (130 ku) was detected (Figure 4). These results were consistent with RT-PCR results of human gastric adenocarcinoma cell lines.

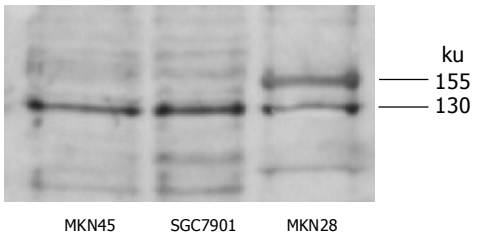

Figure 4 Detection of protein of VLDLR subtypes (160 and 110 ku) in MKN28, SGC7901, and MKN45.

DISCUSSION

Since VLDLR was cloned in 1992 and found to have its subtypes, there have been more and more researches about the distribution and function of VLDLR subtypes[2-5]. The cause of VLDLR subtype formation is the result of the alternative splicing for 16 exon. Yet, tissues and cells per se do not contain VLDLR genes that express two VLDLR subtypes respectively. *O*-linked sugar region is the only distinction between two VLDLR subtypes, and plays an important role in enhancing VLDLR stability and binding capacity with ligand[4,15]. It is well known that, compared to type II VLDLR, type I VLDLR has relatively stronger affinity to VLDL and β-VLDL and is more stable, while both subtypes have no significant differences in internalization, dissociation, and degradation of ligand[4,16]. It has been believed that the function of VLDLR is mainly to bind lipoproteins including apoE and participate in triglyceride metabolism. Now some studies have found that this receptor functions as a signal receptor on the membrane, binding many ligands, inducing extracellular transport and signal transduction of signal molecule, and then producing many biological effects[17-19]. However, the functional difference of VLDLR subtypes, especially the distinct function and signification of type II VLDLR has still not been defined yet.

The previous researches showed that the expression level of VLDLR subtypes changes during the development of embryonic tissues in mouse or chicken, indicating that it is correlated with tissue growth and development[6-9]. Particularly, Nakamura *et al*[20,21] found that type II VLDLR is the major receptor in the early phase of fetal brain development, and that type I VLDLR mainly presented in adult brain, both of which are related to brain development. Furthermore, the recent research suggested that in the embryogenesis of brain, VLDLR and apoER$_2$ may induce the signal transduction between extracellular Reelin and intracellular Disabled-1 (Dab1), which phosphorylates Dab1 and activates intracellular kinase to affect the migration and accurate localization of

developed neuron[22,23]; type II VLDLR is possibly the important receptor binding Reelin in developed brain and inducing Reelin signal transduction pathway[20]. However, after adipocytic 3T3-L1 cells were induced to differentiate into adipocyte-like cells, the expressions of both VLDLR subtypes have a concomitant increase, especially for type I VLDLR[24]. This is significantly beneficial for adipocyte to absorb and store triglyceride. Therefore, the variations of VLDLR subtype expression fairly reflect the requirement of special function during various phases of tissue growth and development. In addition, we observed previously that the expression level of VLDLR subtypes also changes in some pathologic tissues[25]. For example, type II VLDLR in spleen and adipose tissues disappeared from the patients with liver cirrhosis and in adipose tissues of patients with diabetes did not have obvious changes, yet the mRNA level of type II VLDLR significantly decreased or disappeared[25], implying that there might be some linkages between special pathological changes. Thereby, according to the tissue-specific distribution of VLDLR subtypes and the changes mentioned above, VLDLR subtypes, especially type II VLDLR, are likely to have other important functions besides lipid metabolism.

Our research firstly found that type I and II VLDLR are differentially expressed in various differentiated gastroenteric carcinoma tissues and cells. In particular, type II VLDLR is mainly expressed in poorly- or moderately-differentiated gastroenteric carcinoma. The mechanism and/or cause of these variations remains unknown. In addition, type II VLDLR also predominates in fetal normal intestinal epithelial, gastric adenocarcinoma cell (AGS) and many adenocarcinomas such as adenocarcinoma of the breast, gallbladder, lung, colon, ovary and parotid gland[14]. It has recently been found that VLDLR's ligands such as tissue factor pathway inhibitor (TFPI), urokinase-type plasminogen activator-type-1 and plasminogen activator inhibitor complex (uPA-PAI-1) play some roles in tumor proliferation, differentiation, infiltration and metastasis by different signal pathways, respectively[26-32]. The phenomenon strongly showed that type II VLDLR is closely related to the occurrence, differentiation and progress of carcinoma. The VLDLR subtype expressing changes in variedly differentiated carcinoma are different in their respective functions in tissue growth and development. The variations of VLDLR subtypes in differentiated cancer cell may be caused by several aspects, namely, the different ability to bind the same or different ligands, the relevant signal pathways, and the subsequent biological effects. VLDLR subtypes can adapt or regulate the differentiation of cancer cells, and simultaneously produce different effects on the differentiation and proliferation of cancer cells. In addition, the construction of cytoskeleton needs lots of phospholipids during cell differentiation and proliferation, where type II VLDLR may play an important role in myelin intake[21]. These might be the causes of the different expression of VLDLR subtype at various differentiations of cancer cells. However, whether the variation of VLDLR subtype expression might be a mark of cancer differentiation and whether it could be used as a clinical evidence of early diagnosis and treatment for cancer, needs to be further investigated.

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