Significance of LRP and PPAR-γ Expression in Lipomatous Soft Tissue Tumors

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Abstract: Background: Molecular mechanism of differentiation in lipogenic tumor is still unknown in detail. Low-density lipoprotein receptor-related protein (LRP) and peroxisome proliferator-activated receptor gamma (PPAR-γ), representative regulatory molecules of lipogenic differentiation, have been reported today as multi-functional molecules and to modulate tumorigenesis in various kind of cancer. To date, diagnostic and therapeutic significance of the expression of these molecules in lipogenic tumors are not defined.

Methods: The immunohistochemical expression status of LRP and PPAR-γ in various grades of 54 lipogenic tumors was analyzed. Correlation between the expression levels and the differentiation of the tumors was confirmed. For statistical analyses, the Kruskal-Wallis test, the Steel-Dwass test and the Mann–Whitney U test were used.

Results: LRP and PPAR-γ expression was detected in 50 (92.6%) and 44 (81.5%) cases, respectively. The expression level in LRP was significantly higher in cases with well differentiated liposarcoma, pleomorphic liposarcoma and dedifferentiated liposarcoma than in lipoma. Compared with lipoma or well differentiated liposarcoma, significant elevation in expression level of PPAR-γ was confirmed in myxoid liposarcoma, pleomorphic liposarcoma, dedifferentiated liposarcoma and the differentiated area of dedifferentiated liposarcoma.

Conclusion: The up-regulation of LRP and PPAR-γ in higher grade cases, i.e. less differentiated tumors than in low grade cases was shown, suggesting the candidate role of these molecules as tumor progression modulators rather than regulatory molecules of differentiation in lipogenic tumors.

Keywords: Lipogenic tumor, LRP, PPAR-γ, immunohistochemistry.

INTRODUCTION

Low-density lipoprotein receptor-related protein (LRP) is a cell surface receptor that is frequently found in the liver, placenta, brain, epithelial cells of the digestive system, smooth muscle cells, macrophages and fibroblasts [1-3]. LRP is a member of the low-density lipoprotein receptor (LDLR) family, which comprises LRP, LDLR, very low-density lipoprotein receptor and other several molecules [4]. When LRP was originally identified, the structural similarities to LDLR and expression in the liver suggested a role in lipoprotein metabolism and cholesterol homeostasis. Further in vitro evidence that LRP binds apolipoprotein E (apo E) led to the proposal that this molecule serves as a receptor for chylomicron remnants, lipoproteins that primarily shuttle dietary cholesterol from the gut to the liver. However, considerable evidence began to emerge suggesting that a major function of LRP lies in the removal of proteinase and proteinase inhibitor complexes, raising the possibility that this molecule acts as a multifunctional scavenger receptor [4]. To date, LRP has been reported to bind and endocytose over 30 structurally and functionally distinct ligands, including proteases such as matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (uPA) [4]. The importance of these proteolytic enzymes in tumor progression suggests a critical role for this molecule in malignancy. In fact, LRP expression has been reported in various kinds of tumors, including astrocytoma, colon cancer, renal cancer and melanocytic tumor [5-8]. However, the precise roles and potential underlying mechanisms for this molecule in malignancy remain controversial.

Peroxisome proliferator-activated receptors (PPARs) are members of a super family of nuclear receptors. The PPAR subfamily has three isotypes: α, β/δ, and γ, which are ligand-activated transcription regulators that are important in cellular homeostasis [9]. Among these, PPAR-γ is a nuclear hormone receptor that plays a critical role in adipocyte differentiation and control of lipid uptake [10]. The molecule is expressed in preadipocytes at limited levels and is turned on during
Significance of LRP and PPAR-γ Expression in Lipomatous Soft Tissue Tumors

MATERIALS AND METHODS

Specimens were obtained from 54 patients who had undergone surgical resection or open biopsy at the registered institutes. Histological diagnosis was confirmed by standard light-microscopic evaluation of sections stained with hematoxylin and eosin by two authors (Y.F. and F.K.). Classification of tumors used in this study was based on the revised World Health Organization (WHO) criteria for soft tissue tumors with minor modification [19]. Tumors that did not have enough volume for making 5 sections, all 5 most-typical sections in each case. If the samples of cases did not have enough volume for making 5 sections, all sections were evaluated. Immunohistochemical staining for antigens was evaluated using a modified method of Nawa et al. [10, 22]. In brief, tumor cells showing a definite staining pattern were scored as positive, regardless of staining intensity. Areas containing the largest number of positive cells were selected and numbers of positive cells per 600 tumor cells were counted. Percentages of positive cells were calculated as the positive rate. If the number of cells failed to reach 600, the number of positive cells as a percentage of the total countable tumor cells was calculated as the positive rate. For dedifferentiated cases, the well-differentiated area and dedifferentiated area were evaluated separately. For statistical analyses, the Kruskal-Wallis test, Steel-Dwass test and Mann-Whitney U test were used. Values of P<0.05 were considered significant. All patients were informed that the surgical specimen would be subject to this study, and provided written form consent.

This study was approved by the institutional ethics committees, and informed consent was obtained from each patient.

RESULTS

Using a monoclonal antibody directed against the LRP, we found LRP in the lipomatous tumor of 50 patients (92.6%) (Fig. 1). No immunostaining was observed after the omission of primary antibody (data not shown). Significant differences in LRP were confirmed among 7 groups. The positive rate was significantly higher in WDLS, PLLS and DDLS than in lipoma. No significant differences were identified between WDLS and lipoma. Compared with the expression rate in lipoma and WDLS, a significant elevation in positive rate was confirmed in MLS, M/RLS, PLLS, DDLS and the area of WDLS in DDLS. Interestingly, a considerably high expression rate was confirmed in both DDLS and the area of WDLS in DDLS.
Fig. (1). Immunohistochemical expression of LRP in lipoma (b), WDLS (d), M/RLS (f), PLLS (h), DDLS (j) and DDLS-WDLS (l). Negative control (normal mouse serum) of each sections (a, e, g, i, k). Original magnification ×400. Positive expression was seen in 50 patients (92.6%).
Correlations of expression rates between these two molecules were also analyzed, confirming a significant correlation between the two molecules ($p = 0.004$) (Fig. 5).

**DISCUSSION**

The present study has shown the up-regulation of LRP and PPAR-$\gamma$, both of which are believed to be critical regulatory molecules of lipogenic differentiation, in high-grade, i.e., less-differentiated lipogenic tumors, than in low-grade tumors.

The regulatory function of LRP on tumor was identified on the basis of the following; distinct expression in various malignant tumor; ligation with MMP or uPA, which are critical for tumor invasion [4-8]. However, the actual role of this molecule in malignancy, i.e., whether inhibitory or progressive, remains controversial due to variations in the relationship between expression level and tumor grade [6, 7, 8, 23]. The origin of such controversial aspects is, at least partly, thought to arise from the multi-functional properties of this molecule [22, 24]. Variety of parameters might change the role of this molecule in regulating tumor activity, resulting in controversial aspects of the properties of the molecule. In the interpretation of the molecular expression in lipogenic tumor, multifactorial functions of the molecule both in terms of fat metabolism and differentiation and of regulatory activity of tumor should have been considered. We first planned this study under the hypothesis that the molecule could regulate fat differentiation in lipogenic tumor, i.e. higher expression in low-grade or benign cases. However, the results revealed an inverse trend. LRP might thus act as a tumor progression regulator rather than in the differentiation of the fat lineage in the system. Previously, up-regulation of uPA expression has been noted in the invasive front or cases of myxoid liposarcoma with worse prognosis (Morii T, et al. unpublished data in English literature), suggesting a close relationship between LRP and tumor progression.

A recent report has suggested a regulatory function of PPAR-$\gamma$ ligands for LRP expression [15]. Interestingly, a significant relationship between expression rates was identified between these two molecules in the present study, supporting the above-mentioned hypothesis. Further systemic analysis of the proteolysis-internalization system comprising LRP and related proteolytic enzymes, together with PPAR-$\gamma$ function in regulating LRP in terms of both tumor activity and fat differentiation, should be performed in the future.

Overexpression of PPAR-$\gamma$ in high-grade cases or malignancy has been reported [25-28]. In vitro analyses showing that antagonism of this molecule results in cell growth inhibition support these results [13, 25]. In contrast, other reports have found PPAR-$\gamma$ to be expressed more often in cases showing low grade or better prognosis, rather than in those with high grade or poor prognosis [20, 29]. In the present study, expression of PPAR-$\gamma$ was shown to be elevated according to the malignancy of the lipogenic tumor. A previous report has confirmed the expression and up-regulation of m-RNA of this molecule in lipogenic tumor [10]. However, that report did not show the relationship of expression intensity and tumor grade or differentiation, perhaps because of a smaller number of cases, unlike the present study. Up-regulation of this molecule in high-grade and less-differentiated cases has evoked several enigmas as to the function of this molecule in lipogenic tumor, because this molecule was, at least originally, believed to have a regulatory function in fat differentiation. As with LRP, we initially hypothesized that down-regulation might be confirmed in less-differentiated cases.
Fig. (3). Immunohistochemical expression of PPAR-γ in lipoma (b), WDLS (d), M/RLS (f), PLLS (h), DDLS (j) and DDLS-WDLS (l). Negative control (normal mouse serum) of each sections (a, c, e, g, i, k). Original magnification ×400. The positive expression was seen in 44 patients (81.5%).
Significance of LRP and PPAR-γ Expression in Lipomatous Soft Tissue Tumors

Fig. (4). Average PPAR-γ-positive rate. Results are shown as mean and standard deviation (SD). DDLS-WDLS: Area of WDLS in dedifferentiated liposarcoma. * \( P<0.05 \): Positive rate was significantly higher in MLS, PLLS, DDLS and WDLS in DDLS than in lipoma. # \( P<0.05 \): Positive rate was significantly higher in MLS, M/RLS, PLLS, DDLS and WDLS in DDLS than in WDLS.

Fig. (5). Correlation of expression rate between LRP and PPAR-γ \( (p=0.004, R^2=0.135) \).
PPAR-γ also has multiple functions, including acting as a promoter of lipogenesis, inflammation regulator and tumorigenesis promoter. PPAR-γ ligands such as troglitazone induce growth arrest not only in macrophages and endothelial cells, but also in several cancer cells such as prostate and breast cancer cells both in vitro and in vivo [30, 31]. Where expression is elevated in low-grade cases, some authors have attributed the phenomenon to activation of the ligand-receptor system that suppresses tumor activity [20]. This hypothesis seems plausible, but most reports of PPAR-γ expression in malignancy have revealed the converse, supporting the results of the present study. Although the precise reasons for this discrepancy are difficult to clarify, some authors have speculated that considering the diversity of human cancer, expression of PPAR-γ may be dependent on tissue specificity and/or the mutational events requisite for cancer development [26, 32].

Although still under debate in some aspects, the role of PPAR-γ in tumor growth inhibition has been extensively studied in terms of experimental, clinical and etiological aspects during the last several years. Many experimental model studies have demonstrated that PPAR-γ ligands are anti-tumorigenic due to anti-proliferative, pro-differentiation and anti-angiogenic effects [13, 25, 33-35]. Moreover, etiological analysis on a database from ten Veteran Affairs medical centers in the United States revealed a strong association between the use of pioglitazone and reduced risk of lung cancer [36].

The present data suggested the possibility of clinical application of these immunohistochemical methods as strong tool for the evaluation of malignancy in lipogenic tumors. Tumors with high expression of PPAR-γ can thus be candidates for molecular targeted therapy through the application of PPAR-γ ligands that can suppress tumorigenesis or tumor proliferation. In fact, a recent clinical trial showed the probability of this receptor as a target of tumor suppression in liposarcoma [10, 37], suggesting tumor suppression in patients with intermediate/high-grade liposarcoma through the induction of differentiation by the PPAR-γ ligand. The subject in the present study was consistent with the largest range of lipomatous tumors and showed a broad range of considerably higher expression for this receptor in high-grade cases than in low-grade or benign cases, supporting the potential of this approach as a promising and attractive strategy.

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REFERENCES

[1] Boucher P, Gotthardt M. LRP and PDGF signaling: A pathway to atherosclerosis. Trends Cardiovasc Med 2004; 14(2): 55-60.

[2] Moestrup SK. The alpha 2-macroglobulin receptor and epithelial glycoprotein-330: Two giant receptors mediating endocytosis of multiple ligands. Biochim Biophys Acta 1994; 1197(2): 197-213.

[3] Moestrup SK, Gliemann J, Pallesen G. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. Cell Tissue Res 1992; 269(3): 375-82.

[4] Herz J, Strickland DK. LRP: A multifunctional scavenger and signaling receptor. J Clin Invest 2001; 108(6): 779-84.

[5] Obermeyer K, Krueger S, Peters B, et al. The expression of low density lipoprotein receptor-related protein in colorectal carcinoma. Oncol Rep 2007; 17(2): 361-7.

[6] Desrosiers RR, Rivard ME, Grundy PE, Annabi B. Decrease in LDL receptor-related protein expression and function correlates with advanced stages of Wilms tumors. Pediatr Blood Cancer 2006; 46(1): 40-9.

[7] Yamamoto M, Ikeda K, Ohshima K, et al. Increased expression of low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor in human malignant astrocytomas. Cancer 1997; 89(13): 2799-805.

[8] de Vries TJ, Verheijen JH, de Bart AC, et al. Decreased expression of both the low-density lipoprotein receptor-related protein/alpha(2)-macroglobulin receptor and its receptor-associated protein in late stages of cutaneous melanocytic tumor progression. Cancer Res 1996; 56(6): 1432-9.

[9] Michalik L, Desvergne B, Wahli W. Peroxisome-proliferator-activated receptors and cancers: complex stories. Nat Rev Cancer 2004; 4(1): 61-70.

[10] Tontonoz P, Singer S, Forman BM, et al. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. Proc Natl Acad Sci USA 1997; 94(1): 237-41.

[11] Lovell BB. PPARgamma: an essential regulator of adipogenesis and modulator of fat cell function. Cell 1999; 99(3): 239-42.

[12] Chang TH, Szabo E. Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor gamma in non-small cell lung cancer. Cancer Res 2000; 60(4): 1129-38.

[13] Sarraf P, Mueller E, Jones D, et al. Differentiation and reversal of malignant changes in colon cancer through PPARgamma. Nat Med 1998; 4(9): 1046-52.

[14] Mueller E, Sarraf P, Tontonoz P, et al. Terminal differentiation of human breast cancer through PPAR gamma. Mol Cell 1998; 1(3): 465-70.

[15] Gauthier A, Vassiliou G, Benoist F, McPherson R. Adipocyte low density lipoprotein receptor-related protein gene expression and function is regulated by peroxisome proliferator-activated receptor gamma. J Biol Chem 2003; 278(14): 11945-53.

[16] Horvai AE, Schaefer JT, Nakakura EK, O'Donnell RJ. Immunostaining for peroxisome proliferator gamma distinguishes dedifferentiated liposarcoma from other retroperitoneal sarcomas. Mod Pathol 2008; 21(5): 517-24.

[17] Chibon F, Mariotti D, Broët J, et al. A subgroup of malignant fibrous histiocytomas is associated with genetic changes similar to those of well-differentiated liposarcomas. Cancer Genet Cytogenet 2002; 139(1): 24-9.

[18] Rubin BP, Dal Cin P. The genetics of lipomatous tumors. Semin Diagn Pathol 2001; 18(4): 286-93.

[19] Fletcher CDM, Unni KK, Mertens F (Eds.): World Health Organization. Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon (France): IARCPress; 2002.

[20] Papadaki I, Melona E, Giannopouli I, et al. PPARgamma expression in breast cancer: clinical value and correlation with ERbeta. Histopathology 2005; 46(1): 37-42.

[21] Zhu Y, Alvares K, Huang Q, Rao MS, Reddy JK. Cloning of a new family from mouse liver. J Biol Chem 1993; 268(36): 26817-20.

[22] Theocharis S, Giaginis C, Parasi A, et al. Expression of peroxisome proliferator-activated receptor-gamma in colon cancer: Correlation with histopathological parameters, cell cycle-related molecules, and patients’ survival. Dig Dis Sci 2007; 52(9): 2305-11.

[23] Emonard H, Bellon G, de Diesbach P, et al. Regulation of matrix metalloproteinase (MMP) activity by the low-density lipoprotein receptor-related protein (LRP). A new function for an "old friend". Biochimie 2005; 87(3-4): 369-76.

[24] Yoshimura R, Matsumaya M, Segawa Y, et al. Expression of peroxisome proliferator-activated receptors (PPARs) in human urinary bladder carcinoma and growth inhibition by its agonists. Int J Cancer 2003; 104(5): 597-602.
Zhang GY, Ahmed N, Riley C, et al. Enhanced expression of peroxisome proliferator-activated receptor gamma in epithelial ovarian carcinoma. Br J Cancer 2005; 92(1): 113-9.

Sato H, Ishihara S, Kawashima K, et al. Expression of peroxisome proliferator-activated receptor (PPAR) gamma in gastric cancer and inhibitory effects of PPAR gamma agonists. Br J Cancer 2000; 83(10): 1394-400.

Han SW, Greene ME, Pitts J, Wada RK, Sidell N. Novel expression and function of peroxisome proliferator-activated receptor gamma (PPAR gamma) in human neuroblastoma cells. Clin Cancer Res 2001; 7(1): 98-104.

Terasita Y, Sasaki H, Haruki N, et al. Decreased peroxisome proliferator-activated receptor gamma gene expression is correlated with poor prognosis in patients with esophageal cancer. Jpn J Clin Oncol 2002; 32(7): 238-43.

Kubota T, Koshizuka K, Williamson EA, et al. Ligand for peroxisome proliferator-activated receptor gamma (troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo. Cancer Res 1998; 58(15): 3344-52.

Lemberger T, Saladin R, Vazquez M, et al. Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. J Biol Chem 1996; 271(3): 1764-9.

Ikezoe T, Miller CW, Kawano S, et al. Mutational analysis of the peroxisome proliferator-activated receptor gamma gene in human malignancies. Cancer Res 2001; 61(13): 5307-10.

Aljada A, O'Connor L, Fu YY, Mousa SA. PPAR gamma ligands, rosiglitazone and pioglitazone, inhibit bFGF- and VEGF-mediated angiogenesis. Angiogenesis 2008; 11(4): 361-7.

Tanaka T, Kohno H, Yoshitani S, et al. Ligands for peroxisome proliferator-activated receptors alpha and gamma inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res 2001; 61(6): 2424-8.

Oswa E, Nakajima A, Wada K, et al. Peroxisome proliferator-activated receptor gamma ligands suppress colon carcinogenesis induced by azoxymethane in mice. Gastroenterology 2003; 124(2): 361-7.

Govindarajan R, Ratnasinghe L, Simmons DL, et al. Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes. J Clin Oncol 2007; 25(12): 1476-81.

Demetri GD, Fletcher CD, Mueller E, et al. Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma. Proc Natl Acad Sci USA 1999; 96(7): 3951-6.