Annals of Diagnostic Pathology 33 (2018) 11–16

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Peroxisome proliferator-activated receptor γ coactivator-1α is a predictor of lymph node metastasis and poor prognosis in human colorectal cancer

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ARTICLE INFO

Keywords:
PGC-1α
Colorectal cancer
Lymph node metastasis
Prognosis

ABSTRACT

Peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivator-1α (PGC-1α) expression levels are correlated with clinical outcome in breast cancer. However, the potential biological and clinical significance of PPARγ and PGC-1α in colorectal cancer remains unknown. Here we investigated PPARγ and PGC-1α expression in colorectal cancer, and the associations of these expression levels with clinicopathological features. We also evaluated the roles of PPARγ and PGC-1α as prognostic factors in colorectal cancer. We performed immunohistochemical analysis to investigate PPARγ and PGC-1α expression in human colorectal cancer tissues and adjacent normal tissues from 108 primary colorectal cancer patients. We then examined how these expression levels correlated with clinicopathological features. Using the Kaplan-Meier method, we evaluated 3-year disease-free survival (DFS) and overall survival (OS) in patients with tumors expressing different levels of PPARγ and PGC-1α. Our results revealed that PPARγ expression was not significantly correlated with age at surgery, gender, differentiation, depth of infiltration, relapse, or TNM stage. Additionally, PGC-1α expression was not significantly correlated with age at surgery, differentiation, depth of infiltration, relapse, or TNM stage. However, PGC-1α expression was significantly correlated with nodal metastasis ($p = 0.020$). Survival analysis demonstrated reduced OS in the PGC-1α-positive group compared to the PGC-1α-negative group ($p = 0.03$). Our present findings suggest that PGC-1α may be useful for predicting nodal metastasis, and may represent a biomarker for poor prognosis in colorectal cancer.

1. Introduction

Colorectal cancer is among the most common cancers worldwide [1], and its prognosis varies depending on tumor invasion, regional lymph node metastasis, and distant organ metastasis. Recent studies have clarified the sequence of colorectal tumor progression and some of the involved mechanisms [2,3]. However, little is known regarding molecular predictive indicators for regional disease invasion and metastasis. Identification of molecular markers of more aggressive colorectal cancer phenotypes is critical to enable appropriate adjustment of a patient's treatment.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor family [4]. PPARγ heterodimerizes with the retinoid X receptor [5], and this complex binds the PPAR response element to activate transcription of downstream genes [6]. Recent studies identify PPARγ as a putative therapeutic target in a variety of tumor types, based on observations suggesting that PPARγ stimulation may inhibit carcinogenesis and tumor cell growth [7,8]. However, the exact roles of PPARγ in carcinogenesis and tumor cell growth remains controversial, with many conflicting reports indicating that it acts as both a tumor suppressor and tumor promoter [9-11].

Recent investigations of PPARγ-related signaling reveal that this receptor is associated with a group of molecules termed nuclear receptor coactivators [12,13]. In particular, the PPARγ coactivators PGC-1 and PGC-2 form a complex with PPARγ, which collectively binds the basal transcription machineries of target genes, activating transcription. In this manner, nuclear receptor coactivators intervene between receptors and the target genes. The complex of PPARγ with nuclear receptor coactivators associates with transcription factors—such as steroid receptor coactivator-1 (SRC-1) and cAMP response element binding (CREB) binding proteins [14]—and regulates the expression of target genes, such as $p27^{KIP1}$, c-MET proto-oncogene, and E-cadherin. It appears that PPARγ agonists can inhibit cell growth via multiple pathways, including cyclin D1 inhibition [15] and increased levels of the cell cycle inhibitor $p27^{Kip1}$ [16]. PPARγ agonists can also inhibit the transcription of genes involved in tumor progression, such as the HGF receptor c-MET [17].

Real-time reverse transcription polymerase chain reaction (RT-PCR)

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https://doi.org/10.1016/j.anndiagpath.2017.11.007

analyses reveal significantly decreased expression of PGC-1α and PPARγ in colorectal tumors relative to the surrounding normal mucosa [18]. However, PPARγ and PGC-1α expression has no reported biological or clinical implications in colorectal cancer patients, even though PPARγ ligands affect colorectal cancer cell growth [19-21]. The link between PGC-1α and cancer was first indicated by the correlation between poor prognosis in breast cancer and reduced expression of PPARγ and PGC-1α [22]. However, the mechanisms by which PPARγ and PGC-1α affect cell proliferation or differentiation remain unclear, and the biological and clinical significance of PGC-1α in colorectal cancer has not been defined.

In our present study, we investigated PPARγ and PGC-1α expression in 108 primary colorectal cancers and in corresponding samples of normal colorectal tissue. We further examined how the expression of these proteins correlated with clinicopathological factors and prognosis in colorectal cancer patients.

2. Materials and methods

2.1. Patients and tissue samples

This study enrolled 108 consecutive eligible colorectal cancer patients who underwent operations at our University Hospital in 2002–2003. Eligible patients had no family history of colorectal cancer, and had not received preoperative chemotherapy or radiotherapy. Patients were excluded if they had familial adenomatous polyposis or inflammatory bowel disease, synchronous colorectal or extracolorectal cancers, or if they were lost to follow-up. Tissue samples were collected from the enrolled patients, and were formalin-fixed and paraffin-embedded. Information regarding age, sex, histologic grade, and Tumor-Node-Metastasis (TNM) stage [23] was retrieved by reviewing the pathology and surgical reports. After reviewing all of the hematoxylin and eosin (HE)-stained slides from the cases to confirm the original diagnosis, one representative slide was selected from each case for immunohistochemical study. This study was approved by the Institutional Review Board of our University Hospital (approval number 2-104709-AB-N-01-201504-BR-004-02), and written informed consent was obtained.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections were subjected to immunohistochemical analysis for PPARγ and PGC-1α using the avidin-biotin-peroxidase complex method [24]. The primary antibodies were a mouse monoclonal antibody against PPARγ (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a rabbit polyclonal antibody against PGC-1α (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). All sections were deparaffinized as previously described [24]. Immunoreactivity was enhanced by microwave antigen retrieval at 750 W for 30 min in Tris/EDTA buffer (pH 9.0) for PPARγ, and in citrate buffer (pH 6.0) for PGC-1α. Endogenous peroxidase activity was blocked with 5% hydrogen peroxidase for 10 min, and then the samples were incubated with primary antibody for 1 h at room temperature. Subsequently, the secondary antibody reaction, washing, detection, and counterstaining were performed as previously described [24].

All slides were independently evaluated by two independent experienced pathologists who were blinded to clinicopathological data. There were only minor discrepancies in the evaluations, which were resolved by reevaluation under a multi-head microscope until achieving a consensus. The percentage of positive tumor cells and staining intensity (weak, moderate, or strong) were assessed. For PPARγ, immunoreactivity was defined as cells showing nuclear staining with/without cytoplasmic staining patterns in the lesional tissue with minimal background staining. As regards the normal colon tissue adjacent to tumors, tumors with a moderate or strong staining intensity in > 75% of the tumor cells were recorded as PPARγ positive. For PGC-1α, lesions were considered to have immunoreactivity against PGC-1α when > 5% of cells showed moderate to strong positive nuclear staining.

2.3. Statistical analysis

The samples were divided into two groups based on their positive or negative PPARγ and PGC-1α staining. We performed between-group comparisons of the numbers of samples, clinicopathological variables, and patient survival rates. Statistical analysis was performed using Fisher's exact test with a 5% level of significance. Disease-free survival (DFS) was defined as the length of time from surgery to initial disease recurrence, and OS as the length of time from surgery to death or last follow-up. Survival analysis was performed using the Kaplan-Meier method, and statistical significance was evaluated by log-rank test. We used the Cox proportional hazards model to perform multivariate analysis including covariates that showed statistical significance in univariate analysis. A p value of < 0.05 was considered to indicate statistical significance in all analyses. Statistical analyses were performed with SAS 9.4 software.

3. Results

3.1. Expression of PPARγ in human colorectal cancer tissues

When positive staining was observed, moderate to strong PPARγ staining was seen in the nuclei of tumor cells of colorectal cancer and normal colonic mucosa (Fig. 1A–C). Of the 108 patients, 62 (57.4%) were positive for PPARγ nuclear staining, while 46 (42.6%) were negative.

3.2. Relationship between PPARγ expression and clinicopathologic features

Table 1 summarizes the relationship between PPARγ expression and clinicopathological features. Tumor stage was classified according to TNM staging, with 16 patients graded as stage I, 36 as stage II, and 56 as stage III and IV. PPARγ expression was not significantly correlated with age at the time of surgery, gender, tumor location, histopathologic grade, lymph node metastasis, or TNM stage.

3.3. PPARγ expression and outcome in colorectal cancer patients

We used a log-rank test with Kaplan-Meier estimates to assess whether PPARγ expression was a useful prognostic factor for survival among patients with surgically resected colorectal carcinoma. Among the 108 analyzed patients, those positive for PPARγ expression (n = 62) showed a slightly and non-significantly higher 3-year DFS compared to patients negative for PPARγ (69.5% vs. 67.4%, p = 0.5970; Table 3; Fig. 2A). On the other hand, the 3-year OS rate was slightly and non-significantly higher for patients with PPARγ-negative tumors compared to patients positive for PPARγ expression (60.6% vs. 73.1%, p = 0.6878; Table 3; Fig. 2B).

3.4. Expression of PGC-1α in human colorectal cancer tissues

We performed immunohistochemical examinations to assess PGC-1α expression in human colorectal carcinomas. Moderate to strong PGC-1α staining was observed in the nuclei of colorectal tumor cells, whereas no or weak PGC-1α staining was detected in normal colonic mucosa (Fig. 1D–F). In the present study, positive expression of PGC-1α was found in 56 (51.9%) of the 108 colorectal carcinoma tissue specimens, in which the immunostaining occurred predominantly in the nuclei of tumor cells. Nuclear staining for PGC-1α was seen in 37.5% (6 of 16) of stage I tumors, 47.2% (17 of 36) of stage II tumors, and 58.9% (33 of 56) stage III and IV tumors, respectively.