

Original article

Autophagy proteins in prostate cancer: Relation with anaerobic metabolism and Gleason score¹Alexandra Giatromanolaki, M.D.^{a,*}, Efthimios Sivridis, M.D.^a, Savvas Mendrinou, M.D.^c,
Anastasios V. Koutsopoulos, M.D.^a, Michael I. Koukourakis, M.D.^b^a Department of Pathology, Democritus University of Thrace, University General Hospital of Alexandroupolis, Alexandroupolis, Greece^b Department of Radiotherapy/Oncology, Democritus University of Thrace, University General Hospital of Alexandroupolis, Alexandroupolis, Greece^c Division of Pathology Integrated Medical Professionals, New York, NY

Received 3 February 2013; received in revised form 6 April 2013; accepted 7 April 2013

Abstract

Objectives: Up-regulation of autophagy provides an important survival mechanism to normal and malignant cells residing in a hypoxic and unfavorable nutritional environment. Yet, its role in the biology of prostate cancer remains poorly understood.

Methods: In this study we investigated the expression of four major autophagy proteins, namely the microtubule-associated protein 1 light chain 3A (LC3A), LC3B, Beclin 1, and p62, together with an enzyme of anaerobic metabolism, the lactate dehydrogenase 5 (LDH5), in relation to Gleason score and extraprostatic invasion. A series of 96 prostate adenocarcinomas was examined using immunohistochemical techniques and appropriate antibodies.

Results: The LC3A protein was expressed in the form of “stone-like” structures, and diffuse cytoplasmic staining, the LC3B reactivity was solely cytoplasmic, whereas that of p62 and LDH5 was both cytoplasmic and nuclear. A median count of 0.90 “stone-like” structures per 200× optical field (range 0–3.6) was highly associated with a high Gleason score. Similarly, a strong cytoplasmic LC3A, LC3B, and p62 expression, when extensive (present in >50% tumor cells per section), was significantly associated with LDH5 and a high Gleason score. In addition, extensive cytoplasmic p62 expression was related with LC3A and B reactivity and also with extraprostatic invasion. Extensive Beclin-1 expression was significantly linked with extraprostatic invasion and also with p62 and LDH5 expression.

Conclusions: Immunohistochemical detection of autophagy proteins may potentially prove to be useful as prognostic markers and a tool for the stratification of patients in therapeutic trials targeting autophagy in prostate cancer. © 2014 Elsevier Inc. All rights reserved.

Keywords: Autophagy; LC3A; LC3B; p62; LDH5; Prostate cancer

1. Introduction

Autophagy is a fundamental cellular process through which waste intracellular material, such as damaged or aged proteins and organelles, is eliminated and, subsequently, recycled for energy production [1]. This highly complex phenomenon is initiated by signaling molecules, such as the proteins Beclin 1 (autophagy-related gene [Atg] 6 protein) and ULK1/Atg1, that drive the assembled Atg proteins to form autophagic membranes that mature to autophagosomes [2,3]. The MAP1-LC3/Atg8 (A-C) are the essential

structural proteins of the autophagosomes and these are widely used as markers for studying the autophagy kinetics [4]. The transport of impaired proteins and organelles to autophagic membranes, which occurs well before the maturation of the spheroid autophagosomes, is mediated by specific carriage proteins, such as the p62/sequestosome 1, that bind to ubiquitinated proteins and mitochondria and bring them to the inner surface of the LC3 positive autophagosomes [5]. The final step of the autophagic machinery is the fusion of the autophagosomes with lysosomes, and the degradation of the autophagic content by lysosomal enzymes.

An abnormal autophagic function tends to interfere with the quality of cellular proteins leading to a variety of degenerative disorders, cell dysfunction, and often cancer development [6,7]. In experimental studies, up-regulation of

* Corresponding author. Tel.: +255-1-075-117; fax: +255-1-030-349.

E-mail address: agiatrom@med.duth.gr (A. Giatromanolaki).

¹The study was financially supported by the Tumor and Angiogenesis Research Group.

Table 1

Details of the antibodies, dilutions, and antigen-retrieval methods used in this study

Primary antibody	Dilution/ incubation time	Antigen retrieval	Specificity	Source
AP1805a	1:200 (30 min ^a)	MW	LC3A	Abgent, San Diego, CA
LC3-5F10	1:100 (overnight ^b)	MW	LC3B	NanoTools, Teningen, GE
Ab64134	1:200 (30 min ^a)	MW	p62	Abcam, Cambridge, UK
Ab51031	1:50 (30 min ^a)	MW	Beclin 1	Abcam, Cambridge, UK
Ab9002	1:200 (overnight ^b)	MW	LDH5	Abcam, Cambridge, UK

MW = microwave heating.

^aAt room temperature.

^bAt 4°C.

the autophagic process renders cancer cells either sensitive or resistant to irradiation or chemotherapy or both, suggesting a rather complicated role of autophagy in the response of cellular to cytotoxic agents [8]. On the contrary, autophagy-related proteins are highly expressed in a variety of human tumors and often linked with aggressive behavior, as this was shown in a number of previous studies [8–11].

Although targeting autophagy, as a means of therapeutic intervention, is under intense investigation for several malignancies, including prostate cancer [13–15], the expression of autophagy-related proteins in this latter tumor has not been examined in the past. In this study, a series of 96 prostate adenocarcinomas was investigated for the expression of important autophagy proteins, the microtubule-associated protein 1 light chain 3A (LC3A), the LC3B, and the p62; in addition, the anaerobic enzyme lactate dehydrogenase 5 (LDH5)—an isoenzyme composed of 5 LDHA subunits—was examined. The above proteins were related to Gleason score and to extraprostatic invasion.

2. Materials and methods

A series of 96 node negative prostate adenocarcinomas was stained immunohistochemically for the expression of the autophagic proteins LC3A, LC3B, and p62, as well as the LDH5 isoenzyme. The material was retrieved from the files of the Department of Pathology, Democritus University of Thrace Medical School, Alexandroupolis, Greece from patients who had undergone radical prostatectomy at the University General Hospital. The material consists of consecutive surgical samples received between 1995 and 2003. The median age of patients was 67 years (range 44–88). The Gleason score ranged from 5 to 10 (median 6). Of the 96 adenocarcinomas, 65 had a score <7 (67.7%) and 31 (32.3%) had a score ≥7. Invasion of the prostatic capsule or the seminal vesicles or both was detected in 19 (19.8%) cases.

2.1. The immunohistochemical procedure

The antibodies against the autophagic proteins LC3A and LC3B were chosen among a panel of eight commercial antibodies after western blot analysis (data not shown; study under submission); this was considered necessary to secure specific detection of LC3A and LC3B as several commercially available antibodies recognize these proteins.

2.1.1. Immunohistochemistry for LC3A, Beclin 1, and p62

The automated Bond-max system (Leica Microsystems) was used for the detection of these proteins in malignant and in normal/hyperplastic prostate specimens. Sections were cut at 3 µm from formalin-fixed paraffin-embedded tissues and dried for 30 minutes at 80°C. The slides were then covered by Bond Universal Covertiles (Leica Microsystems) and placed into the Bond-max instrument. This was followed by deparaffinization of tissue on the slides with Bond Dewax Solution (Leica Microsystems) at 72° for 15 minutes; heat-induced antigen retrieval with Bond Epitope Retrieval Solution 2 (Leica Microsystems) for 30 minutes at 100°C; peroxide block placement on the slides for 5 minutes at room temperature; and incubation with the primary antibodies (Table 1). The subsequent steps of the procedure included incubation with post primary reagent (Leica Microsystems) for 10 minutes at room temperature; bond polymer (Leica Microsystems) placement on the slides for 10 minutes at room temperature; development of color reaction in 3,3'-diaminobenzidine (DAB) tetrahydrochloride for 10 minutes at room temperature; section counterstaining with hematoxylin for 5 minutes at room temperature; and, finally, mounting the slides.

2.1.2. Immunohistochemistry for LC3B

Sections, 3 µm thick, were deparaffinized and placed in an antigen-retrieval target solution pH 9.0 (DAKO), followed by microwaving (3 × 5 min). The primary monoclonal antibody to LC3B (Table 1) was applied at a dilution of 1:100 overnight at 4°C. Following washing with phosphate-buffered saline (PBS), endogenous peroxidase was quenched with EnVision Flex Peroxidase Block (DAKO) for 10 minutes. It was followed by washing with PBS. Nonspecific binding was blocked in EnVision Flex mouse Linker for 15 minutes (DAKO), and washed in PBS. Sections were then incubated with a secondary antibody (EnVision Flex/HRP; DAKO) for 30 minutes, and washed in PBS. The color was developed within 5 minutes of incubation with DAB solution, and sections were counterstained weakly with hematoxylin.

2.1.3. Immunohistochemistry for LDH5

Sections, 3 µm thick, were deparaffinized and placed in an antigen-retrieval target solution pH 9.0 (DAKO), followed by microwaving (3 × 5 min). Nonspecific binding was blocked in normal rabbit serum at a dilution of 1:20 for 30 minutes (DAKO, X0902). No rinsing was performed. The primary

Download English Version:

<https://daneshyari.com/en/article/6194473>

Download Persian Version:

<https://daneshyari.com/article/6194473>

[Daneshyari.com](https://daneshyari.com)