Expression Profile of Autophagy-related Markers in Localized Prostate Cancer: Correlation With Biochemical Recurrence After Radical Prostatectomy

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学位論文の内容要旨

Expression Profile of Autophagy-related Markers in Localized Prostate Cancer: Correlation With Biochemical Recurrence After Radical Prostatectomy

限局性前立腺癌におけるオートファジー関連マーカー発現プロファイル：根治的前立腺全摘除術後の生化学的再発との相関性

神戸大学大学院医学研究科医科学専攻

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Summary

INTRODUCTION

Radical prostatectomy (RP) has been the mainstay of treatment for localized prostate cancer (PC); however, biochemical recurrence (BR), defined as a persistent elevation of serum prostate-specific antigen (PSA), develops in approximately 30% of men undergoing RP. Nevertheless, PC has been shown to exhibit unique biological features and markedly heterogeneous genetic backgrounds, indicating the limitations for predicting postoperative prognostic outcomes in men with localized PC solely using conventional clinicopathologic parameters.

Autophagy, a lysosome-dependent pathway for protein degradation, is characterized by the formation of double-membrane-bound organelles known as autophagosomes. The dysregulation of autophagy has been demonstrated to be involved in various pathologic conditions. In recent years, there have been several studies reporting that the expression profiles of molecular markers consisting of the autophagy pathway could be used as useful predictors of clinical courses in several types of human malignant tumor; however, limited information is available on the prognostic significance of autophagy-related proteins in patients with PC.

Considering these findings, we evaluated the expression patterns of multiple autophagy-related proteins, including autophagy-related gene 5 (Atg5), autophagy-related gene 9 (Atg9), Beclin1, microtubule-associated protein light chain 3 (LC3), and UNC-51-like kinase 1 (ULK1), in RP specimens from 160 patients with localized PC to analyze the prognostic significance of these markers in this cohort of patients.

METHODS

This study included a total of 160 consecutive patients who were diagnosed with clinically organ-confined PC and then underwent RP and bilateral pelvic lymphadenectomy without any neoadjuvant therapies. Expression levels of 5 autophagy markers, including autophagy-related gene 5, autophagy-related gene 9, Beclin1, microtubule-associated protein light chain 3, and ULK1, in radical prostatectomy specimens from 160 consecutive patients with clinically localized PC were measured by immunohistochemical staining.

RESULTS

Of these 5 markers, ULK1 expression was significantly correlated with the incidence of BR. On univariate analysis, ULK1 expression, serum prostate-specific antigen level, pathologic stage, Gleason score, seminal vesicle invasion, and surgical margin status were identified as significant predictors of BR. All these significant factors except for seminal vesicle invasion were independently associated with BR on multivariate analysis. Furthermore, significant differences in BR-free survival according to the positive numbers of these 5 independent risk factors were noted, that is, BR occurred in 2 of 33 patients negative for risk factors (6.1%), 20 of 76 patients positive for 1 or 2 risk factors (26.3%), and 38 of 51 patients positive for ≥3 risk factors (74.5%).

COMMENT

Autophagy is regarded as an important cellular process, through which waste intracellular materials, such as aged or damaged proteins and organelles, are digested and recycled for renewed energy production. This complex process is conducted by a group of evolutionarily conserved proteins, including the 4 following functional groups: Atg proteins mediating the induction of autophagy (Beclin1, ULK1,

Atg13, and Atg17), vesicle nucleation (ULK1, Atg13, and Atg17), autophagosome formation (Atg5,Atg12, and LC3), and retrieval of autophagy-related proteins (Atg2, Atg9, and Atg18). Despite intensive efforts to clarify the molecular mechanism of autophagy, its functional role during the progression of human malignancies remains controversial. Furthermore, inconsistent findings have been reported with respect to whether the expression profile of autophagy-related markers is associated with the prognosis of patients with malignant tumors. In the field of PC research as well, a few studies assessed the significance of molecular markers involved in autophagy. For example, Giatromanolaki et al reported that the strong and extensive expression of LC3 in PC specimens was closely associated with the Gleason score, whereas Jiang et al showed the significant impact of the elevated expression of a mitochondrion-associated autophagy inhibitor, leucine-rich pentatricopeptide repeat motif-containing protein, on the prognosis of PC patients at a late stage with a poor prognosis. To our knowledge, however, there have not been any studies investigating the prognostic impact of molecular markers involved in the autophagic pathway for men with early stage PC.

In this series, BR occurred in 37.5% of the included patients, and the 5-year BR-free survival rate was 63.2%, which is consistent with those reported in previous studies. Furthermore, immunohistochemical studies revealed that various expression levels of the 5 autophagy-related proteins were detectable, even at very low levels, in the majority of PC tissues. Based on precise assessment of the expression level of each marker in RP specimens considering both the staining area and its intensity, the expression level of ULK1 was shown to be significantly correlated with the incidence of BR. In addition, ULK1 expression appeared to have a significant impact on serum PSA

level, but not on Gleason score. These findings suggest the crucial role of ULK1 in regulation of the disease extension of PC, rather than that of biological aggressiveness, at an early stage.

The precise prediction of the postoperative clinical course of patients undergoing surgical resection of malignant tumors is important for determining the appropriate postoperative follow-up schedule, as well as additional therapeutic options. In patients with PC, a number of studies have been carried out to identify factors, including molecular markers, predicting the biochemical outcome after RP, and several types of nomograms for calculating the probability of BR after definitive therapy targeting localized PC have been reported. Accordingly, we subsequently examined the impacts of potential markers on the autophagy pathway, in addition to those of several clinicopathologic parameters, on the BR-free survival after RP. Univariate analysis showed that postoperative BR was significantly correlated with the serum PSA level, pathologic stage, Gleason score, SVI, SMS, and expression levels of ULK1. Of these factors, the serum PSA level, pathologic stage, Gleason score, SMS, and ULK1 expression were identified as independent predictors of BR-free survival on multivariate analysis. The prognostic significance of the 4 independent conventional parameters in patients with PC undergoing RP has been well recognized; however, this may be the first study to demonstrate the potential impact of ULK1 expression on the postoperative prognosis of patients with PC. Considering these findings, it is necessary to compare the present finding with other molecular markers and nomograms and to conduct additional analysis of PC specimens, including genomic assessment to develop a more precise system to predict the prognosis of PC patients after RP.

It is of interest to consider the theoretical background behind the unfavorable impact of ULK1 overexpression on the postoperative prognosis of patients with PC. ULK1, one of the major human autophagy-related genes with a serine-threonine kinase activity, forms a stable complex with other autophagy-associated proteins. The high-performance mechanisms to initiate autophagy are very important to organisms when cells undergo stress under normal conditions, and the ULK1 complex is the core of the autophagy induction machinery. Taken together, in cancer cells, particularly those in early-stage disease, a high level of ULK1 expression may contribute to activate the initiation of autophagy and protect them from apoptosis, which could explain the present findings showing unfavorable biochemical outcomes after RP in patients with a strong expression of ULK1. However, there have been several studies presenting conflicting outcomes regarding the expression level of ULK1 as a prognostic marker in cancer patients. Collectively, these findings suggest that ULK1 may have different roles in different types of cancer; thus, it is necessary to intensively investigate the impact of ULK1 expression on the prognosis of patients with a wide variety of malignant tumors.

Another point of interest is to develop a system to more precisely predict the biochemical outcome after RP by combining potential prognostic indicators. Based on the outcomes achieved by multivariate analysis, the 160 patients were divided into the following 3 groups according to the positive number of independent risk factors for BR: negative for any risk factor, positive for 1 or 2 risk factors, and positive for ≥3 risk factors, and there appeared to be significant differences in BR-free survivals among these 3 groups. It is necessary to strictly evaluate whether the improved prediction of

BR by additional investigation of autophagy-related markers by immunohistochemical staining justifies the effort and cost when compared with the use of conventional clinicopathologic factors alone; these findings suggest that a simultaneous consideration of the 5 major risk factors identified in this series may contribute to develop a novel system that can more accurately predict BR after RP.

CONCLUSION

Collectively, these findings suggest that measurement of expression levels of potential autophagy markers, particularly ULK1, in RP specimens, in addition to conventional parameters, may contribute to the accurate prediction of BR after RP for localized PC.

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論文審査の結果の要旨			
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審査委員 Examiner	主　査 Chief Examiner 掛地吾弘 副　査 Vice-examiner 眞庭謙昌 副　査 Vice-examiner 井上健一		

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CONCLUSION

Collectively, these findings suggest that measurement of expression levels of potential autophagy markers, particularly ULK1, in RP specimens, in addition to conventional parameters, may contribute to the accurate prediction of BR after RP for localized PC.

The candidate, having completed studies on the accurate prediction of biochemical recurrence after radical prostatectomy, with a specialty in autophagy marker ULK1, and having advanced the field of knowledge in the area of prostate cancer, is hereby recognized as having qualified for the degree of Ph. D. (Medicine).