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授与した学位	博　士
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学位授与の要件	医歯薬学総合研究科病態制御科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Dynamic contrast-enhanced MRI as a predictor of programmed death ligand-1 expression in patients with oral squamous cell carcinoma (口腔扁平上皮癌患者における Programmed death ligand-1 発現の予測因子としてのダイナミック造影 MRI の応用)
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学位論文内容の要旨

Recent years witnessed the approval of immune checkpoint inhibitors (ICIs) for the treatment of patients with recurrent/metastatic head and neck squamous cell carcinoma (HNSCC) and who experience disease progression after treatment with a platinum-based chemotherapy agent. These ICIs, namely nivolumab and pembrolizumab, aim at targeting an important immunological checkpoint in HNSCC and oral squamous cell carcinoma (OSCC), which is the programmed death-1 receptor (PD-1)/programmed death ligand-1 (PD-L1) interaction. Nivolumab and pembrolizumab block the inhibitory interaction between PD-1 and PD-L1, which reactivates the immune system and enhances tumor cell elimination. PD-L1 expression is commonly used as a biomarker to predict the therapeutic effect and response rates to ICIs. PD-L1 expression is assessed through biopsy. Although biopsy is common for confirmation of disease recurrence, it is an invasive procedure and may in some cases be inaccessible due to difficult location. Therefore, considering a complementary and minimally invasive procedure for assessing PD-L1 expression might be beneficial for patients when making a treatment plan.

Magnetic resonance imaging (MRI) is a widespread imaging technique that is frequently used for the diagnosis of HNSCC. In particular, dynamic contrast-enhanced (DCE)-MRI produces functional images, which led to an increased number of studies investigating the potential of DCE-MRI in the assessment of histopathological features. Microvessel density (MVD) is a key histopathological feature. MVD reflects the intensity of angiogenesis within the tumor and can be assessed following vessels staining with the endothelial cell marker CD31. Previous studies reported that DCE-MRI parameters correlate with MVD in patients with OSCC, and that MVD correlates with PD-L1 in patients with classical Hodgkin lymphoma. Based on these studies, we speculated that DCE-MRI parameters might be associated to PD-L1. This study aimed to ascertain the reported correlation between DCE-MRI and MVD and to investigate whether DCE-MRI can be used to noninvasively assess PD-L1 expression.

Twenty-one patients with primary OSCC who underwent 3T MRI, including DCE-MRI, at Okayama University Hospital, were included in this study. For each lesion, the region of interest (ROI) was drawn on DCE-MRI images. The signal intensity (SI) and the contrast index (CI) of each ROI were calculated. The time course of the CI was then plotted to obtain a CI curve. Using the CI curve, the following DCE-MRI parameters were defined: CI-max,

T-max, CI-peak and CI-gain.

The MVD, vascular endothelial growth factor (VEGF) expression and PD-L1 expression in the surgically resected specimens were analyzed using immunohistochemistry (IHC) staining for CD31, VEGF and PD-L1, respectively. The MVD was defined as the number of microvessels in a microscopic field of 0.67 mm2. The samples were sorted into low and high MVD groups based on the median MVD. VEGF, expressed as a percentage, was defined as the number of positively stained cells to the total number of cells. The samples were sorted into low and high VEGF expression groups based on the median VEGF expression. PD-L1 expression was semi-quantified using the tumor proportion score (TPS). Based on the TPS, the samples were sorted into three groups: Negative (<1%), low-positive (1-49%) and high-positive (50-100%) PD-L1 expression.

Statistical analyses were performed using the non-parametric tests Spearman's correlation, U-Mann Whitney test and Kruskal Wallis test. The correlations between the DCE-MRI parameters, the MVD, VEGF expression and PD-L1 expression were determined. Comparisons of DCE-MRI parameters according to the MVD levels and PD-L1 expression levels were also determined.

The Results of the present study showed that the median MVD, which was used to divide the patient cohort into low (n=10) and high (n=11) MVD groups, was 49.25. The MVD showed a strong negative and statistically significant correlation with T-max ($r=-0.61$, $P=0.003$) and a moderate positive and statistically significant correlation with CI-gain ($r=0.46$, $P=0.037$). The mean T-max was significantly shorter, and the mean CI-gain was significantly higher and in high-MVD tumors ($P<0.01$ and $P<0.05$, respectively).

The median VEGF expression, which was used to divide the patient cohort into low (n=10) and high (n=11) VEGF expression groups, was 75.10. VEGF expression showed a non-statistically significant correlation with the DCE‐MRI parameters, MVD and PD-L1 expression.

For PD-L1 expression, the patient cohort was divided into negative (n=9), low-positive (n=6) and high-positive (n=6) PD-L1 expression groups. PD-L1 expression showed moderate positive and statistically significant correlations with CI-max ($r=0.57$, $P=0.007$), CI-peak ($r=0.57$, $P=0.007$), and CI-gain ($r=0.58$, $P=0.006$). PD-L1 expression also showed a strong positive and statistically significant correlation with MVD ($r=0.66$, $P=0.001$). The mean CI-max, CI-peak, CI-gain, and MVD were significantly higher in tumors with high-positive PD-L1 expression ($P<0.05$).

In summary, DCE-MRI parameters were associated with MVD. These findings support that DCE-MRI may be used to assess MVD and be able to distinguish tumors with low and high levels of angiogenesis. Furthermore, DCE-MRI parameters were associated with PD-L1 expression on tumor cells. Thus, DCE-MRI may be used to assess PD-L1 expression and identify tumors with high-positive PD-L1 expression. The findings of the present study led to the assumption that DCE MRI may predict response to anti-angiogenic therapy and to anti-PD-L1 therapy in patients with OSCC.

論文審査結果の要旨

【Introduction】The present study intended to provide a new approach to noninvasively assess programmed death ligand-1 (PD-L1) expression in patients with oral squamous cell carcinoma (OSCC). For that purpose, the value of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was investigated.

【Methods】For the study, images of 21 patients diagnosed with primary OSCC who underwent DCE-MRI were collected and examined. Quantitative DCE-MRI parameters were derived from the signal intensity (SI) and the contrast index (CI) curves. The respective surgically resected specimens were used for immunohistochemical (IHC) examination. The expression of CD31, a marker that stains vascular endothelial cells and thereby highlights blood vessels, was used to assess microvessel density (MVD). CD31 expression for MVD and PD-L1 expression were then correlated with the DCE-MRI parameters.

【Results】As a first outcome, the present study verified the previously reported association between DCE-MRI and MVD. The DCE-MRI parameters; T-max and CI-gain; were significantly correlated with MVD. As a second and fundamental outcome, the present study investigated the assumption that DCE-MRI may be able to assess PD-L1 expression. DCE-MRI parameters; CI-max, CI-peak and CI-gain; were found to be significantly correlated with PD-L1 expression on tumor cells.

【Discussion】The present study affirms the notion that the enhancement pattern observed on DCE-MR images is influenced by the intratumor angiogenesis. According to this study, DCE-MRI may be a valuable method to noninvasively assess angiogenesis in OSCC. The substantial research finding is that DCE-MRI can identify patients with high-positive PD-L1 expression. This may be of a great value considering that high-positive PD-L1 tumors have conceivably a favorable response to immune checkpoint inhibitors (ICIs). DCE-MRI may thus help to determine patients who will benefit from treatment with ICIs.

This paper highlights the prognostic and predictive value of DCE-MRI as a noninvasive diagnostic imaging tool assessing the tumor behavior using PD-L1 expression. Therefore, DCE-MRI could be indicated as an adjunctive examination to improve ICIs treatment. This paper has been published in Oncology Letters and has been evaluated internationally. The defense committee hereby accept this article as a doctoral dissertation in dentistry.