学 位 論 文 の 要 旨

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学　位　論　文　名: Applicability of Preoperative Nuclear Morphometry to Evaluating Risk for Cervical Lymph Node Metastasis in Oral Squamous Cell Carcinoma

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論 文 内 容 の 要 旨

INTRODUCTION

We previously reported the utility of preoperative nuclear morphometry for evaluating risk for cervical lymph node metastases in tongue squamous cell carcinoma. The risk for lymph node metastasis in oral squamous cell carcinoma, however, is known to differ depending on the anatomical site of the primary tumor, such as the tongue, gingiva, mouth floor, and buccal mucosa. In this study, we evaluated the applicability of this morphometric technique to evaluating the risk for cervical lymph node metastasis in oral squamous cell carcinoma.

MATERIALS AND METHODS

Data collection

Data were retrospectively collected for patients who were histopathologically diagnosed with OSCC and underwent surgical management including neck dissection at the Department of Oral and Maxillofacial Surgery, Nagasaki University Medical and Dental Hospital between January 1986 and January 2001 and the Department of Oral and Maxillofacial Surgery, Shimane University Faculty of Medicine between 1981 and 2012. Recurrent cases were excluded.

Biopsy specimens and pathologic nodal classification

Biopsy was performed in all patients preoperatively and/or prior to neoadjuvant therapy. The biopsy specimens were fixed with 10% neutral buffered formalin for 24 h and were processed for routine paraffin embedded sections, then stained with hematoxylin and eosin.

All the lymph nodes dissected from the biopsy specimens were examined for pN status and level of the metastatic lymph nodes. Cervical lymph node level was determined based on the cervical lymph node metastatic guide.

Image analysis and nuclear parameter measurements

Images of each section were stored using a standard light microscope (using x10 objective lens) connected to a computerized digital camera. The image data were analyzed by Mac Scope software (Mitani Co., Fukui, Japan) to estimate the various quantitative nuclear features (at least 100 nuclei per case). Nuclear margins were digitally marked under high power view on the computer screen to ensure measurement accuracy.

Mean (standard deviation) values of the nuclear area and perimeter were calculated from counts of the pixels capturing the nuclei and their edges. The nuclear circular rate and aspect ratio were automatically calculated to determine variations in shape; briefly, in a round circle, the circular rate and aspect ratio values correspond to 1: if the object is elliptical, the circular rate is <1 and the aspect ratio is >1. NACV was calculated to express variations in size in individual cases. The study protocol was approved by the Ethics Committee of Shimane University Hospital (Approval No.: 1286).

Statistical analysis

To examine differences in patient characteristics between pN-positive and pN-negative patients, we performed *t*-tests for continuous variables. *p* values less than 0.05 were considered statistically significant.

Logistic regression analysis was performed to identify the risk factors for node-positive status. Odds ratios and confidence intervals (based on the Wald test) were also calculated. Candidate risk factors with *p* values less than 0.1 on the Wald test were selected. From among them, risk factors were determined by variable selection using Akaike's Information Criterion. The optimal cutoff values for measurements were obtained with the minimum *p* values from the *t*-tests. All analyses were performed using SAS version 9.3 software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Eighty-eight cases of squamous cell carcinoma (52 of the tongue, 25 of the gingiva, 4 of the buccal mucosa, and 7 of the mouth floor) were included: 46 with positive node classification and 42 with negative node classification. Nuclear area and perimeter were significantly larger in node-positive cases than in node-negative cases; however, there were no significant differences in circular rate, aspect ratio, or NACV. We derived two risk models based on the results of multivariate analysis: Model 1, which identified age and mean nuclear area and Model 2, which identified age and mean nuclear perimeter. It should be noted that primary tumor site was not associated the pN-positive status. There were no significant differences in pathological nodal status by aspect ratio, NACV, or primary tumor site.

A simple and reliable method for evaluating the preoperative risk for lymph node metastasis would be indispensable in routine clinical practice, and our approach requires no special equipment or staining technique. Several papers discuss that nuclear shape is a critical factor in the characterization of many neoplastic and non-neoplastic proliferations, and irregularity of the nuclear shape is one of the morphological characteristics commonly used to determine the type or degree of neoplastic transformation. Size and contour irregularities of the nuclei are important features in the grading of OSCC. Furthermore, it was reported that the nuclear size was larger in proportion to the grade of malignancy.

On the whole, among the quantitative morphometric parameters of the nuclei analyzed in this study, the results suggest that malignant nuclei become aspherical, which would be consistent with previous reports. Regarding the patient's age, however, it has been reported that low age (<42 years) was associated with the development of cervical lymph node metastasis within a short time frame (≤50 days). In the present study, age<65 years was suggested to be a risk factor for cervical lymph node metastasis in OSCC.

Future studies also need to address the applicability of clinical and biological marker analyses to accurately evaluate metastatic potential in OSCC preoperatively. Recently, the nuclear factor kappa B was reported to be a key protein in multi-step carcinogenesis, lymph node metastasis, and prognosis of oral, head, and neck squamous cell carcinoma. The expression of a combination of nuclear factor kappa B or other markers should be examined further.

CONCLUSION

Our method of preoperative nuclear morphometry may contribute valuable information to evaluations of the risk for lymph node metastasis in oral squamous cell carcinoma.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

頸部リンパ節転移は，口腔扁平上皮癌（OSCC）の重要な予後因子であり，頸部リンパ節郭清は治療上も重要な意味を持つが，術前に転移の有無を評価することは非常に困難である．リンパ節転移の評価にはDNA解析等いくつかの報告があるが，いずれも手技が煩雑であるため簡便かつ再現性の高い術前予測法の確立が望まれる．申請者のグループは，過去に病理学的に確認された頸部リンパ節転移例pN(+)と非転移例pN(-)では，原発巣の核形態に違いがあることを見出しており，本研究では，客観的核異型度解析法によるOSCCの頸部リンパ節転移の予測法の確立と，リンパ節転移のリスク因子を明らかにすることを目的とした．

対象は，島根大学医学部附属病院歯科口腔外科および長崎大学病院口腔外科にて頸部郭清術（一部リンパ節生検）が施行されたOSCC 88例（男性56例:女性32例，平均年齢64.6歳，舌52例:歯肉25例:頬粘膜4例:口腔底７例）である．分化度は，高分化型65例，中分化型19例，低分化型4例であった．また， pN(+)症例は46例，pN(-)は42例であった．生検標本（HE染色）画像をパソコンに取り込み，Mac Scope softwareにて，5項目（核面積と周囲長，円形度，針状比ならびに核面積変異係数）を解析した．核解析結果に対し，pN(+)とpN(-)症例間でt検定を行った．さらに，解析結果，年齢，性別，分化度，原発部位等すべての項目でロジスティック回帰解析を行った．

その結果，核面積と周囲長はpN(+)症例で有意に高値を呈した．円形度はpN(+)で小さく，針状比はpN(+)症例で大きく，また核面積変異係数はpN(+)症例で大きかったが，いずれも統計学的有意差は認めなかった．ロジスティック回帰解析では，核異型度解析結果にカットオフ値（核面積：80.3μm²，核周囲長：32.7μm）が見出せた．また，臨床的な因子のうち，年齢（65歳未満）も頸部リンパ節転移のリスク因子になることが明らかとなったため，これらを組み合わせれば，頸部リンパ節郭清の術式決定に寄与することが示唆された．今後はPET-CT等による術前検査に本研究結果を併せ，口腔癌における頸部リンパ節転移の有無を前向きに調査するプロジェクトが企画されている．

最終試験又は学力の確認の結果の要旨

申請者は生検組織における口腔扁平上皮癌細胞の核形態計測を客観的に行い，核面積と核周囲長が頸部リンパ節転移のリスク因子となることを見出した．結果はリンパ節郭清の術式決定に有用な結果と考えられ，審査にあたっては質疑応答も的確であり，関連知識も豊富であることから学位授与に値すると判定した．　（主査 丸山理留敬）

申請者は多数の口腔癌の臨床病理学的な検討を行い，口腔扁平上皮癌の核の形態学的な特徴を定量的に評価することで，頸部リンパ節への転移リスクを判定できる可能性を示した．関連領域の知識も豊富で博士の学位に値すると判定した．　（副査 木下芳一）

申請者は口腔癌の頸部リンパ節転移の予測が，術前の腫瘍生検標本の核異型度を画像解析装置を用いて測定することで可能になることを示した．口腔癌の手術成績を改善させうる重要な所見であり，学術的価値の高い研究である．また申請者は質問にも的確に答え，学位授与に値すると判断された．　（副査 鈴宮淳司）

（備考）要旨は、それぞれ４００字程度とする。