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The role of apelin in the assessment of response to chemotherapy and prognosis in stage 4 nonsmall cell lung cancer

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Background/aim: Prediction of response to chemotherapy and prognosis bears clinical significance in patients with lung cancer. The aim of the study was to examine the association between apelin expression in tumor tissues and overall survival, progression-free survival, chemoresistance, and treatment response in stage 4 nonsmall cell lung cancer (NSCLC) patients undergoing chemotherapy.

Materials and methods: A total of 81 patients who received chemotherapy due to a biopsy-documented diagnosis of NSCLC between 2004 and 2011 were retrospectively studied. Bronchoscopic biopsy samples were examined immunohistochemically.

Results: Of the overall study population (n = 81), the mean age was 59.0 ± 9.2 years; 83% (n = 67) were male and 17% (n = 14) were female. All patients received chemotherapy. A total of 30 patients (37%) had no apelin positivity, while 21 (30%) had 1 +, 20 (25%) had 2 +, and 10 (12%) had 3 + apelin positivity. We detected no association between apelin positivity and overall survival, 6-month survival, or 1-year survival rates (P = 0.05, 0.74, and 0.63). Patients with apelin expression as compared to those without it had shorter overall survival (P = 0.05).

Conclusion: Our results suggest that apelin, an angiogenic factor, does not seem to provide significant prognostic information in this patient group.

Key words: Nonsmall cell lung cancer, apelin, prognosis

1. Introduction

Lung cancer has become the leading malignancy afflicting human beings since 1985, in most part due to the global increase in smoking rates (1), and is responsible for 12.8% of all cancer cases. It is responsible for most cancer deaths, i.e. 17.8%, worldwide (2). For all lung cancer patients, only 15% live more than 5 years after diagnosis. Lung cancer incidence decreases in women corresponding to an increase in men (3).

The most important factor in the etiology of lung cancer is smoking. Smoking is responsible for approximately 85%–90% of lung cancer (4). Smoking increases the risk of lung cancer by 30 times compared to nonsmokers (5). Passive smoking increases the risk by about two times (6). Another important factor that played a role in the development of lung cancer in Turkey is asbestos exposure. Smokers being in contact with asbestos increases the risk of lung cancer by 90 times (7).

Nonsmall cell lung cancer (NSCLC) represents approximately 85% of all lung cancers (8). At presentation, 85% of patients have stage 3 or 4 disease, rendering chemotherapy the first line of treatment option. However, cure is not possible with chemotherapy in stage 4 disease (9). Studies focusing on adjuvant chemotherapy since 1995 have only provided a very limited 5-year survival advantage in the order of 5% (2). Due to the presence of significant interpatient variability in terms of response rates to chemotherapy, prognostic predictors for specific chemotherapy regimens are of clinical importance. This will not only allow avoiding unnecessary toxicity from chemotherapy, but will also facilitate customized treatment for each patient. Thus, in recent years there has been a dramatic increase in the number of studies examining the role of molecular markers in the prediction of prognosis and response to treatment in patients with lung cancer. In earlier studies, it was shown that high ERCC1 levels in

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NSCLC patients are an independent prognostic predictor rather than low ERCC1 levels (10). The presence of K-ras mutation has poor prognostic significance for survival in patients (11).

Apelin is a peptide secreted from the cell surface of various tissues including lungs, heart, kidneys, adipose tissue, and breast tissue that functions as a ligand for G proteins. The apelin receptor was originally described by O'Dowd et al. (12) and consists of a 77-amino acid prepropeptide. Proteases are responsible for the formation of apelin-13, apelin-17, and apelin-36 through cleavage from the C-terminal. Among these isoforms with different activities, the shortest isoform is the most potent activator of the apelin receptor (APJ), and it is found in the endothelial cells responsible for the embryogenic development of the vascular system. Apelin and its receptor can be detected in high concentrations in the endothelial cells of the vascular wall in adults (13). Apelin also plays different roles in a number of physiological processes such as fluid hemostasis, regulation of food intake, cell proliferation, glucose utilization, and angiogenesis. In humans and mice, apelin has been found to be produced by adipocytes, which is an indication of the fact that apelin is an adipokine (14).

Currently, apelin is considered to be an angiogenic factor similar to VEGF, and its potential role in limiting tumor angiogenesis is being studied. One third of human cancers produce apelin, which stimulates tumor growth (15). In experimental studies with mice, increased APJ mRNA has been detected, with APJ expression in all peripheral tissues. Higher detection rates in lung and heart tissues have also been found (16). The site of tissue apelin expression is determined by vascular endothelial cells, adipose tissue, and epithelial cells. In mouse embryos, APJ expression has been detected in endothelial cells of the newly developing blood vessels (17). The apelin/APJ system triggers vascular endothelial cell proliferation through the induction of cell-to-cell interactions. Currently, there is a search for agents that are able to inhibit tumor angiogenesis, and in this regard regulation of APJ activity may allow the development of molecules that can normalize vascular growth patterns. Such agents may increase the therapeutic efficacy through the vessel dilating effects of antiangiogenic drugs. APJ and apelin immunohistochemical images are shown in human umbilical vena endothelial cells in Figure 1 (18).

The primary objective of this study was to examine the association between apelin expression in tumor tissues overall, as well as progression-free survival, and to compare response rates for the assessment of chemoresistance in stage 4 NSCLC patients undergoing chemotherapy. Secondary endpoints included the assessment of the association between apelin positivity and side effects of chemotherapy.

2. Materials and methods

2.1. Study population and protocol

We examined patients who received chemotherapy in the Department of Pulmonary Medicine due to a biopsy-documented diagnosis of NSCLC between 2004 and 2011 retrospectively. Patients were included in the study if adequate information on disease stage, response to chemotherapy, side effects, and prognosis was present in patient files. In the case of lacking data on overall side-effect profile, hematological side effects were recorded, if present.

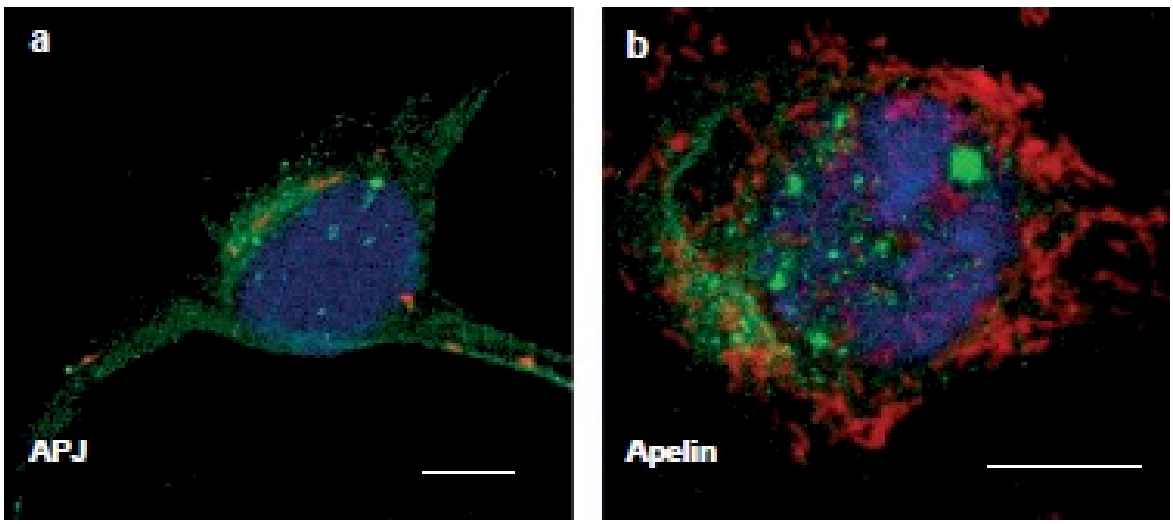

Figure 1. Immunohistochemical images of APJ and apelin in human umbilical vena endothelial cells, from Kleinz and Davenport (18).

A total of 81 NSCLC patients with adequate amounts of tissue sample for an immunohistochemical examination of apelin were included. All patients underwent transnasal or transoral fiberoptic bronchoscopy (Olympus CLE-10) after local anesthesia with topical lidocaine HCl 2% (max. 10 mL). Diagnosis was based on biopsy results in all patients and biopsy samples were assessed histopathologically before treatment. The total number of chemotherapy courses, chemotherapeutic agents administered, response to chemotherapy during follow-up, and hematological side effects of chemotherapy were recorded and prognosis was evaluated. Overall survival was defined as the time from diagnosis to death, and progression-free survival was defined as the time from the start of chemotherapy to the first day of progression. Survival analyses were based on the last day of the study follow-up period. We used CTCAE (Common Terminology Criteria for Adverse Effects) for the assessment of side effects occurring during the course of chemotherapy. The study protocol was approved by our institutional ethics committee.

2.2. Immunohistochemical examinations

Cross-sections of 5 μm in thickness, obtained from paraffin blocks containing adequate amounts of tumor tissue, were placed on electrostatically charged microscope slides and were dried at 60 °C for at least 2 h. All immunohistochemical staining procedures, including deparaffinization and antigen exposure, were performed in a fully automatic immunohistochemical staining device (BenchMark XT). The primary antibody, i.e. Anti-Apelin-12, was administered manually by instillation and incubated for 32 min at 37 °C.

Staining in the bronchial covering epithelium and glandular epithelial tissue was used as a positive control. Cross-sections where the primary antibody could not be instilled were taken as negative controls. Apelin immunoreactivity was graded using the method proposed by Berta et al. (18), whereby the proportion of cells with cytoplasmic positivity was taken into account. Accordingly, 0 staining, 1 + staining, 2 + staining, or 3 + staining (as shown in Figure 2) categories were defined on the basis of no staining, 1% to 10% staining, 11% to 50% staining, and greater than 50% staining, respectively.

2.3. Assessment of the response

We assessed the response to treatment by CT or PET-CT after the 2nd, 3rd, 4th, or 6th chemotherapy courses at the discretion of the treating physician. Treatment response was assessed using the RECIST 1.1 criteria (Response Evaluation Criteria in Solid Tumors).

2.4. Statistical analyses

The study data were analyzed using SPSS 20.0 for Windows. Clinical and pathological characteristics were compared using the Fisher exact test and chi-square test. Overall survival and disease-free survival were evaluated using Kaplan–Meier and log rank (Mantel–Cox) methods. The comparison for mean age and time to progression was performed using the t-test. $P < 0.05$ was considered significant.

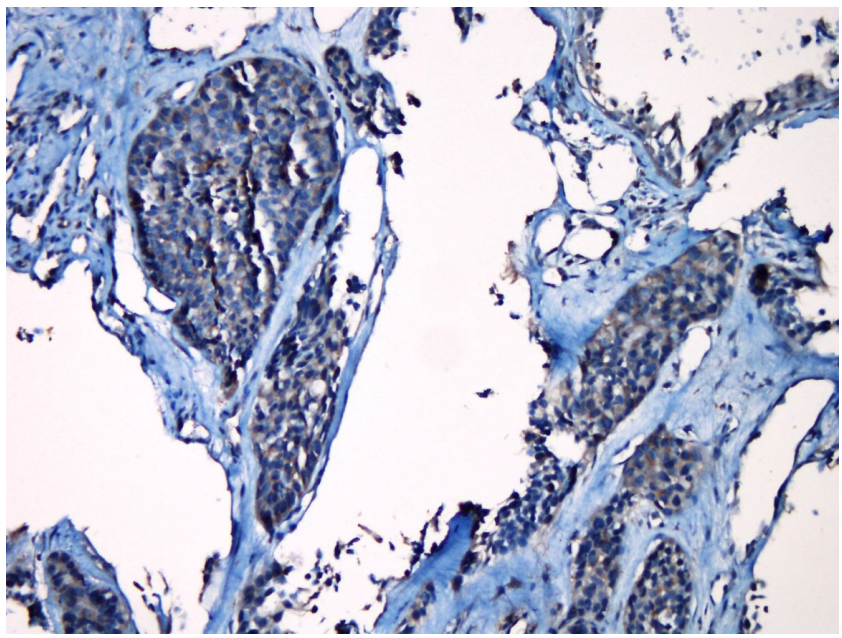

Figure 2. Apelin score of 3 (Anti-Apelin, 100×).

3. Results

The mean age of the study participants was 59 ± 9.2 years. Of the study subjects, 83% (n = 67) were male and 17% (n = 14) were female. A total of 68 patients (84%) had a history of cigarette smoking, with an average smoking history of 51.0 ± 30.0 pack-years. Basic clinical and pathological characteristics of patients are shown in Table 1. Forty-two patients (52%) had at least one concomitant medical condition, the most frequent of which were as follows: hypertension (12 patients, 15%), chronic obstructive pulmonary disease (9 patients, 11%), diabetes mellitus (10 patients, 12%), and coronary artery disease (8 patients, 10%). Only 7% (6 patients) had a family history of cancer.

Diagnostic subcategories included squamous cell carcinoma in 30 patients (37%), adenocarcinoma in 20 patients (25%), and undefined NSCLC in 31 patients (38%). All patients had stage 4 NSCLC. The most frequent site of distant metastasis was the lungs in 24 patients (30%), followed by bone in 22 patients (27%) and the brain in 15 patients (19%). Multiple metastases were present in 31 patients (38%). While progression was observed only in the lungs in 49 patients (61%), progression was observed at the site of metastasis in 11 patients (14%), and 12 patients (15%) had progression both in the lungs and at the site of metastasis. In 8 cases (10%) the patient died before an assessment of response; thus, the progression date was taken as the day of death. All patients received chemotherapy, while in 51 patients (53%) radiotherapy was administered at any one point during their follow-up for a number of indications including metastasis, atelectasis, or superior vena cava syndrome.

In first-line chemotherapy, platinum-based chemotherapy was administered to all patients. The most preferred first-line chemotherapeutic regimen was a cisplatin/carboplatin + gemcitabine combination (39 cases, 48%). For second-line chemotherapy, the most preferred chemotherapeutic regimen was single-agent Taxotere. Subgroups were identified by the positivity of apelin. The subgroups were similar in terms of chemotherapy regimens. All patients took antiangiogenic agents and all patients were given tyrosine kinase inhibitors.

Table 1. Basic clinical and pathological characteristics of patients.

Characteristics	Number (%)
Mean age (years)	59.0 ± 9.2
Sex (male/female)	67 (83%) / 14 (17%)
Smoking history (pack-years)	51.0 ± 30.0
Histology	
NSCLC	31 (38%)
Squamous cell	30 (37%)
Adenocarcinoma	20 (25%)
Performance status (ECOG)	
0	20 (25%)
1	47 (59%)
2	12 (15%)
3	1 (1%)

Chemotherapy was postponed in 40 patients (49%) due to severe side effects, while in 11 (14%) dose reduction was required. RBC transfusions were given to 18 patients (22%) before or after chemotherapy, and 1 patient (1%) received a platelet transfusion. The time to progression after first-line chemotherapy was 26.0 ± 16.2 weeks on average. Second-line chemotherapy was given to 44 patients (54%) due to the absence of response to first-line treatment or due to the development of progression during follow-up. Further, 5 patients received third-line chemotherapy.

The mean overall survival was 56.3 ± 4.4 weeks. While 81% of patients (n = 66) survived for at least 6 months, 1-year survival was observed in 43% (35 patients).

Thirty patients (37%) were apelin-negative, while 21 (30%), 20 (25%), and 10 (12%) cases had 1 +, 2 +, or 3 + apelin positivity, respectively. Although a marginally significant association between apelin positivity and overall survival was observed, no significant link between apelin positivity and 6-month or 1-year survival could be detected (P = 0.05, 0.74, and 0.63, respectively). There were also no significant differences between chemotherapy regimens in terms of side effects. Overall survival was shorter in apelin-positive subjects as compared to those without apelin positivity (P = 0.05) (as shown in Table 2 and Figure 3). Most patients without apelin positivity had an ECOG performance status of 0–1 (P = 0.05).

A comparison between patients with different levels of apelin positivity revealed no difference in demographic characteristics as well as in 6-month and 1-year survival rates (P = 0.74 and 0.63, respectively), in chemotherapy regimens administered, and in side effects observed. When apelin 0, 1 +, 2 +, and 3 + patients were categorized into 2 subgroups as reported previously, again no significant differences were found. There were no significant differences between different chemotherapy regimens in terms of side effect profiles. We observed grade II or III hematological toxicities in the overall patient group, and the severity of hematological toxicities did not correlate with apelin positivity.

4. Discussion

Most lung cancer patients already have late-stage disease at the time of diagnosis, rendering chemotherapy the only therapeutic option in the great majority of these subjects. The decision to administer chemotherapy is

Table 2. Comparison of apelin positivity with response and survival.

Characteristics	Apelin = 0 (n = 30)	Apelin ≥ 1 (+) (n = 51)	P-value
Overall survival (weeks)	53.0 ± 8.3	44.0 ± 5.0	0.05
Partial response	10 patients (33%)	14 patients (28%)	0.60
Stable response + progression	20 patients (67%)	37 patients (73%)	0.60
Time to progression (weeks)	27.3 ± 15.1	25.2 ± 17.0	0.60
6-month survival	25 patients (83%)	41 patients (80%)	0.74
1-year survival	14 patients (47%)	21 patients (41%)	0.63

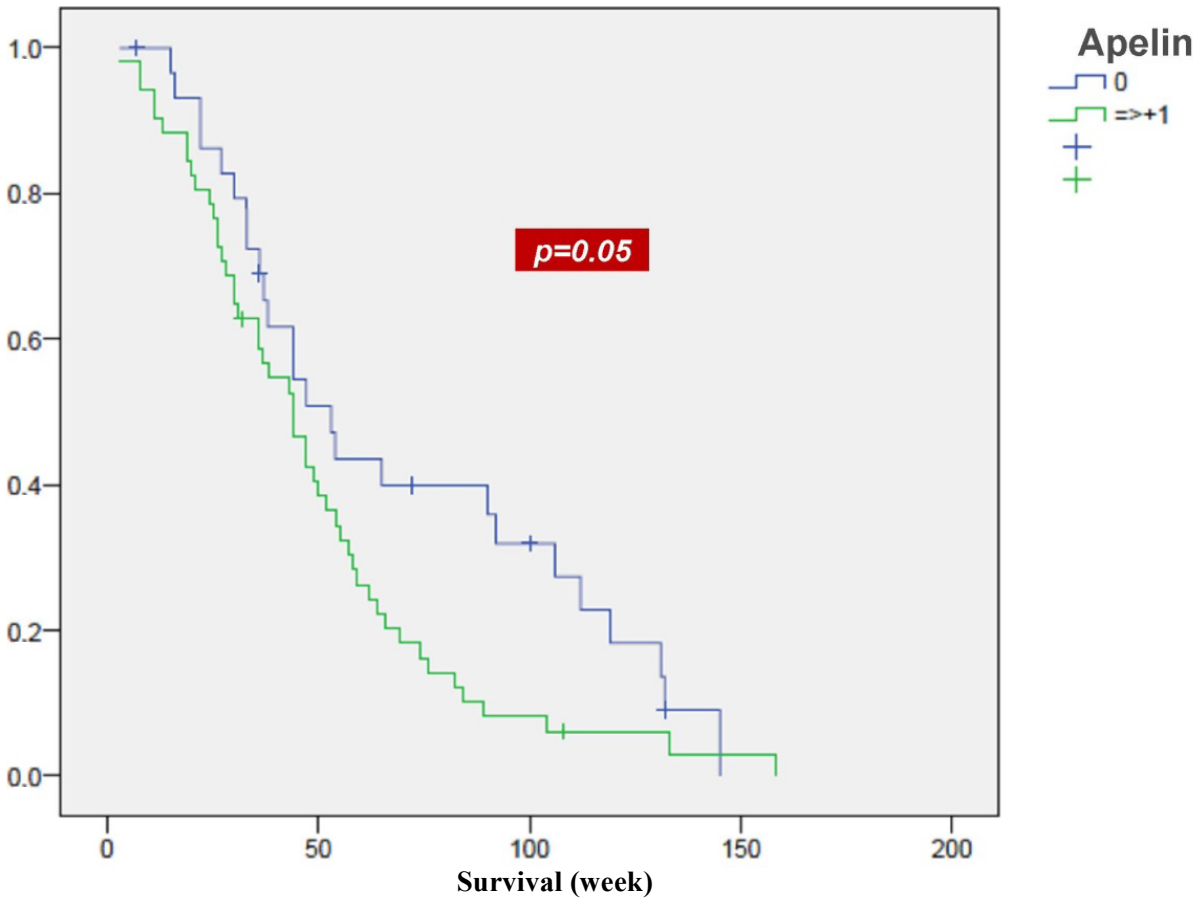

Figure 3. Overall survival curves according to apelin positivity.

based on the performance status and potential side effects. Therefore, prediction of subjects with a greater likelihood of response to chemotherapy using prognostic parameters or determining the subgroup of patients with a higher risk of side effects is of great clinical significance.

In this study assessing the prognostic significance of apelin expression by tumor tissues in advanced-stage NSCLC patients, a marginally significant association between apelin positivity and overall survival was detected, while no such associations could be established for 6-month or 1-year survival rates. Similarly, apelin expression was not associated with differential treatment response rates, different chemotherapy regimens, or hematological side effects.

Our literature search has revealed only a single study examining apelin expression in NSCLC patients (19), where a total of 94 NSCLC patients who received treatment between January 1997 and December 2001

were studied. The mean age of participants was 63 years and the female-to-male ratio was 26:68. All patients were staged on the basis of surgical and pathological findings. Of these patients, 35, 54, and 5 had squamous cell cancer, adenocarcinoma, and large cell cancer, respectively. All patients were evaluated by two independent pathology specialists, and the staining was graded as 0, 1 +, 2 +, or 3 + based on a staining of 0 cells, 1%–10% staining, 11%–50% staining, or greater than 50% staining, respectively.

In the study by Berta et al., 6 different mRNAs were detected for the apelin protein using polymerase chain reaction analysis. Molecular analysis of the tumor and normal lung tissues showed a significantly higher level of apelin mRNA expression within the tumor tissues as compared to normal lung tissues. A significant correlation between mRNA levels and apelin positivity was also observed as detected by immunohistochemical studies (19). Again in the same study, H358 and H1975 cells with endogenously low apelin expression were exposed to an apelin-expressing vector in order to evaluate the effect of apelin on the development of NSCLC, and a significant increase in the expression of apelin by these cells was detected. The apelin-expressing vector was exposed to CD31 antibody in vivo in order to explore the effects of apelin on angiogenesis, and a significant increase in the microvascular density and dimensions of these cells was observed following exposure (19). For the purpose of survival analyses, two groups with different apelin activity, i.e. low apelin expression with 0 or 1 + expression and high apelin expression with 2 + or 3 + expression, were defined. The 5-year survival rate in the low apelin expression group was 63.3%, as compared to 29.9% in the high apelin expression group. Multivariate analysis suggested a prognostic role for apelin expression and lymph node status in NSCLC. These authors concluded that apelin is an angiogenic factor and may represent an independent prognostic parameter associated with poor prognosis that may assist in treatment decisions.

Our study involved only immunohistochemical assessments and a single pathologist evaluated the biopsy material, blinded to patients and treatments. In contrast with the study by Berta et al., only stage 4 patients were included in this study, resulting in shorter survival rates. Our data analysis also showed a shorter survival associated with apelin positivity, although the difference was only marginally significant (P = 0.05). Most of the patients without apelin positivity had an ECOG performance status of 0 or 1 (P = 0.05). The two groups were also similar in terms of demographic data, 6-month survival rate, and 1-year survival rate. In line with previously reported methods, patients with low (0 or 1 + apelin expression) or high (2 + or 3 + apelin expression) apelin expression were also compared, with no significant differences in all parameters tested.

Two additional previous studies tested the prognostic significance of apelin in other organ cancers. The first of these studies involved patients with squamous cell carcinoma of the oral cavity and a significant association between high apelin positivity (3 +) and recurrence rates (P = 0.038) was shown (20). In the second study, apelin expression in the vascular endothelial cells was demonstrated immunohistochemically in patients with breast cancer, but no difference in apelin expression was found between tumor and normal tissues (21).

The major limitations of our study include small sample size and the retrospective nature of our analysis. The principal factor responsible for our small sample size was the inclusion of patients with an adequate amount of biopsy samples both for immunohistochemistry and apelin analysis, who also had to have adequate information in their patient files regarding the stage of cancer, response to chemotherapy, side effects, and prognosis.

In conclusion, our results do not suggest that apelin, an angiogenic factor, may be used as a reliable prognostic factor in this group of patients. Prospective studies with larger sample size are warranted to better elucidate the prognostic role of apelin in that condition.

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