Nerve Growth Factor Links Oral Cancer Progression, Pain, and Cachexia

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Abstract

Cancers often cause excruciating pain and rapid weight loss, severely reducing quality of life in cancer patients. Cancer-induced pain and cachexia are often studied and treated independently, although both symptoms are strongly linked with chronic inflammation and sustained production of proinflammatory cytokines. Because nerve growth factor (NGF) plays a cardinal role in inflammation and pain, and because it interacts with multiple proinflammatory cytokines, we hypothesized that NGF acts as a key endogenous molecule involved in the orchestration of cancer-related inflammation. NGF might be a molecule common to the mechanisms responsible for clinically distinctive cancer symptoms such as pain and cachexia as well as cancer progression. Here we reported that NGF was highly elevated in human oral squamous cell carcinoma tumors and cell cultures. Using two validated mouse cancer models, we further showed that NGF blockade decreased tumor proliferation, nociception, and weight loss by orchestrating proinflammatory cytokines and leptin production. NGF blockade also decreased expression levels of nociceptive receptors TRPV1, TRPA1, and PAR-2. Together, these results identified NGF as a common link among proliferation, pain, and cachexia in oral cancer. Anti-NGF could be an important mechanism-based therapy for oral cancer and its related symptoms. Mol Cancer Ther; 10(9); 1667–76. ©2011 AACR.

Introduction

Pain and cachexia significantly impaire function and degrade quality of life in patients suffering from cancer (1–7). Pain control and weight maintenance are especially challenging in patients with head and neck (e.g., oral) cancer. Many oral cancer patients suffer from symptoms that are more severe than symptoms produced by other cancers (4–7). Oral cancer patients often have trouble eating, drinking, swallowing, and speaking. Despite recent advances in treatment, pain control and weight maintenance persist as 2 important clinical challenges. Cancer pain and cachexia are usually assessed and treated as separate, unrelated entities (1). However, these symptoms may share the same underlying mechanism, because both are linked to chronic inflammation and share common proinflammatory mediators (1, 8). Currently, such a mechanism has not been elucidated for cancer pain and cachexia.

Nerve growth factor (NGF) is a key modulator of the neuroendocrine immune axis that serves diverse biological functions (9–13). In a variety of rodent cancer models, NGF has been shown to play a direct role in tumor proliferation (13–15) and in perineural invasion. Perineural invasion is a predictor of disease progression in oral cancer (15). NGF has also been proposed as an important mediator of tumor-induced bone pain secondary to prostate cancer (16–18). However, the effect of NGF on cancer proliferation and pain is dependent on tumor type. For example, anti-NGF treatment has been shown to reduce disease progression in breast cancer but has no effect in bone cancer (14, 16, 17). Bone cancer pain, however, is attenuated by anti-NGF treatment in mice (16–18). Oral cancer pain is usually caused by cancer originating from soft tissue and clinically distinct from bone cancer pain. Oral cancer pain is exacerbated by function, generally does not arise spontaneously, and is not correlated with tumor size. Even the smallest oral cancers can produce severe pain (4, 5). In contrast, bone cancer pain is commonly spontaneous and incessant, and typically increases with disease progression (16, 17). The effect of anti-NGF on oral cancer pain or proliferation is unknown.

The role of NGF in the regulation of body weight also requires further study. NGF has been shown to participate in glucose and lipid metabolism as well as...
feeding behavior (19). Intraperitoneal injection of NGF stimulates the hypothalamic—pituitary—adrenal axis and causes weight loss in rats (20). Patients with obesity have altered levels of NGF (9, 19, 21). NGF might contribute to inflammation and metabolic disorders associated with body weight changes (19, 21). Similarly, cancer cachexia is a complex wasting syndrome comprising inflammatory and metabolic disturbances (2). Proinflammatory cytokines including TNF-α and interleukin-6 (IL-6) have been shown to induce cachexia by altering metabolism of lipids and muscle proteins (2, 22, 23). NGF modulates the expression and release of these proinflammatory cytokines (10, 24). Accordingly, NGF may play a role in cancer cachexia.

The varied proinflammatory and nociceptive effects of NGF suggest a key role for NGF in cancer symptomatology. We hypothesize that NGF acts as a mediator of proliferation, pain, and cachexia associated with oral cancer. In this study, we first quantified NGF release and expression in patients with oral squamous cell carcinoma (SCC). We then used 2 separate oral cancer models in mice to show that anti-NGF reduces proliferation, pain, and weight loss. Interactions of NGF with proinflammatory cytokines and receptors involved in nociception were also evaluated.

Materials and Methods

Cancer patients

Immunohistochemistry for NGF in human tumors. Oral SCC on the affected side and normal epithelium from an anatomically matched area on the contralateral side of 14 oral cancer patients treated at the University of California San Francisco (UCSF) Department of Oral & Maxillofacial Surgery were obtained. Tissues were fixed with 4% paraformaldehyde (PFA), dehydrated, embedded in paraffin, and cut into 8-μm sections. Microwave antigen unmasking was done by using Dako antigen retrieval solution (Dako). Sections were then incubated with rabbit polyclonal antibodies against NGF (1:100; Serotec, Inc.) for 2 hours. The specificity of NGF antibody was tested and validated in our previous studies (15). Human submandibular gland was used as a positive control as it is known to produce an abundance of NGF. Normal rabbit serum containing mixed immunoglobulins at the concentration of the primary antibody was used as a negative control on the salivary gland tissue specimens. Immunoreactions were visualized with diaminobenzidine chromogen (Vector Laboratories) and counterstained with Mayer’s hematoxylin. This research protocol complies with the Committee on Human Research at the UCSF.

Reverse transcription PCR to quantify NGF mRNA in human tumors. Oral SCC and anatomically matched, contralateral normal oral epithelium from 11 oral cancer patients were surgically removed and immediately snap frozen in liquid nitrogen and stored at −80°C. Total RNA isolation of each sample was conducted with a Qiagen DNA/RNA Kit (Qiagen Inc.) and 1 μL samples were aliquoted for RNA quantitative analysis. Reverse transcription was carried out with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc.) on the biometra thermocycler (template 10 μL volumes per reaction). Quantitative real-time PCR assays were carried out in triplicate with a Taqman Gene Expression Assay Kit (Applied Biosystems Inc.). The housekeeping gene β-gus was chosen as the internal control. Controls consisted of total human brain RNA (~12 ng/μL; Ambion) and were negative in all runs. Relative quantification analysis of gene expression data was conducted according to the 2−ΔΔCt method.

Cell culture of human oral SCC and control keratinocytes. Human oral cancer cells (HSC-3) were cultivated for inoculation into mouse models of cancer pain and proliferation. The HSC-3 SCC cell line was obtained from the Japanese Collection of Research Bioresources (JCRB). The JCRB Cell Bank authenticated the cell line by using short tandem repeat analysis of loci with the PCR-based PowerPlex1.2 system. Once we received the cells from JCRB, the cells were expanded and frozen stocks were prepared. For experiments, cells were used at low passage for not more than 6 months before replenishing with fresh samples from the frozen stocks. Primary normal oral keratinocytes (NOK) harvested from normal oral tissues were cultured as previously described (25).

ELISA quantification of NGF in human oral cancer cells. HSC-3 cells and NOK were grown to confluence and then washed to remove unattached cells. The media for both HSC-3 and NOK were replaced with DK-SFM and incubated for an additional 72 hours. The conditioned media was then removed, centrifuged to remove cell debris, aliquoted, and stored at −20°C. Cells were lysed in cold radiomunoprecipitation assay buffer containing protease inhibitor cocktails (Sigma-Aldrich). The lysate was centrifuged (13,000 rpm for 5 minutes) and the supernatant was removed. ELISA quantification of NGF in human oral cancer tissues was conducted using the NGF Emax Immunoassay ELISA Kit (Promega).

Proliferation assay with anti-NGF in human oral cells. To quantify the effect of NGF on cancer cell proliferation, 3 × 10^3 HSC-3 cells were seeded in individual wells. Control groups received PBS (10 μL) and experimental groups received NGF neutralizing antibody (R&D Systems) in PBS (10 μL of 0.025 μg/mL). At predetermined time points (0, 24, 48, and 72 hours), 20 μL of MTS (Promega Biosciences) was added to each well for 1 hour and incubated at 37°C. The samples were then quantified by MTS colorimetric assay at 490 nm. The experiment was repeated in triplicate.

Mouse cancer models

Behavioral mouse models of human oral cancer pain. Six- to 8-week-old female athymic, immunocompromised mice (BALB/c) were purchased from Charles River Laboratories. They were housed in a temperature-controlled room on a 12:12 light/dark cycle (6 AM to 4
PM light), with *ad libitum* access to food and water. The UCSF Committee on Animal Research approved all procedures and researchers were trained under the Animal Welfare Assurance Program.

**Paw model.** The paw-withdrawal cancer pain mouse model was produced as previously described (26). Adult female nude mice were inoculated with 10⁶ HSC-3 cells in 50 μL of Dulbecco’s Modified Eagle Medium and Matrigel into the plantar surface of the right hind paw.

**Tongue model.** To create a mouse model that is more biologically homologous with human oral cancer, mice were inoculated with 50 μL of 10⁶ HSC-3 cells into the floor of the mouth as previously described (27). The anatomic and functional features of this mouse cancer model parallel those found in human patients with oral cancer (27).

**Anti-NGF treatment and control groups.**

**Paw.** In the mouse paw tumor model, anti-NGF antibody (Mab 256; R&D Systems; 12.5 μg in 20 μL PBS) or vehicle control (20 μL PBS) was injected into the right hind paw of mice starting on postinoculation day, 4 following the pain behavior measurement and twice a week thereafter until postinoculation day 21. Dosage of anti-NGF used was based on a study by Adriaenssens and colleagues (14). Mice were randomly placed into 4 treatment groups: group 1 received an injection of HSC-3 cells and anti-NGF treatment (tumor + anti-NGF, n = 7); group 2 received an injection of HSC-3 cells and PBS (vehicle control, tumor + PBS, n = 7); group 3 received an injection of HSC-3 in the right paw and anti-NGF in the contralateral (CL) paw to see whether anti-NGF has a systemic effect (tumor + CL-anti-NGF, n = 5); group 4 was treated with anti-NGF to determine whether anti-NGF is hypanalgesic in naive mice (naive + anti-NGF, n = 5). All groups of mice were briefly anesthetized with inhalational isoflurane (Summit Medical Equipment Company) during HSC-3 inoculation and drug treatments.

**Tongue.** In the mouse tongue cancer model, 2 groups of mice were used. The control group (n = 10) received isotype immunoglobulin G (50 μg in 50 μL PBS; R&D Systems). The anti-NGF treatment group (n = 10) received 50 μg of the anti-NGF antibody in 50 μL PBS. All injections were intraperitoneal and administered twice per week starting at postinoculation day 13, when all mice exhibited visible tumor masses and increased gnaw time. We were concerned that repeated local injection of anti-NGF into the tongue would affect the rodent’s eating and gnawing behavior so we chose a systemic route of injection (intraperitoneal). Higher doses of systemic anti-NGF were used in the tongue model compared with the dose given in the paw model to ensure enough antibodies reached the tongue tumor.

**Behavioral measurement.** Testing was done by an observer blinded to the experimental groups as previously described (25). The paw withdrawal threshold was measured by an electronic von Frey anesthesiometer (IITC Life Sciences). Paw withdrawal threshold was defined as the force in grams (mean of 8 trials) sufficient to elicit a distinct paw withdrawal flinch upon application of a rigid probe tip.

**Dolognawmeter.** The dolognawmeter is a validated device/assay invented to measure oral function and nociception in mice (27). Mice with tongue tumors were evaluated twice per week with a dolognawmeter as previously described (27). In brief, each mouse was placed into a confinement tube with 2 obstructing dowels in series. The mouse voluntarily gnaws through the 2 dowels to escape from confinement within the tube. Each obstructing dowel is connected to a digital timer. When the dowel is severed by the gnawing of the mouse, the timer is automatically stopped and records the duration of time to sever each of the 2 dowels. To acclimatize the mice and improve consistency in gnawing duration, all mice were trained for 10 sessions in the dolognawmeter. Training involved placing the animals in the device and allowing them to gnaw through the obstructing dowels in exactly the same manner that they do so during the subsequent experimental gnawing trials. A baseline gnaw-time value to sever the second dowel was established for each mouse as the mean of the final 3 training sessions. After baseline gnaw times were established for each mouse, the mice were inoculated with cancer cells.

**Tumor size and body measurement.** Mouse hind-paw volume was measured by using a plethysmometer (IITC Life Science). Tongue tumor volume was calculated at the end of the experiment by multiplying tumor length by width by thickness. Body weight was recorded before and after each behavioral test. Mice did not show any significant weight changes during baseline training trials.

**Tissue and blood processing.** At the end of the experiment, mice with either cancer model were sacrificed with isoflurane. Both tumor and normal paws were dissected and stored at −80°C in preparation for NGF protein quantification. The paw samples were homogenized, lysed, centrifuged, and the supernatant was removed. Total protein concentration in each sample was determined by using a BCA protein assay (Thermo Scientific). NGF concentration was measured by using the same method as previously described. In mice with tongue cancer, blood was rapidly collected from the heart into EDTA-coated tubes. Plasma was separated with a centrifuge and stored at −20°C. Because adipose tissue is the main source of leptin, abdominal fat was also rapidly dissected out and stored at −80°C. Following these manipulations, mice were perfused transcardially with 0.1 mol/L PBS followed by 4% PFA. Trigeminal ganglia were harvested in preparation for sensory receptor and ion channel evaluation. Both trigeminal ganglia and tongues were removed, postfixed in 4% PFA, and cryoprotected in sucrose gradient (20%–50%, 4°C). Serial sections of frozen trigeminal ganglia (10 μm) and tongue (20 μm) were cut on a cryostat and thaw mounted on gelatin-coated slides for processing.

**Immunohistochemistry for sensory receptors and proliferation.** After sectioning, trigeminal ganglia and tongue sections were briefly rinsed in PBS, incubated
in goat serum (5% in PBS with 0.1% Triton X-100) for 1 hour, and then incubated overnight in the primary antibody. Trigeminal ganglia were stained for PAR-2 (goat anti-PAR2, 1:200; Santa Cruz Biotechnology), TRPV1 (rabbit anti-TRPV1, 1:400; Fisher Scientific), and TRPA1 (rabbit anti-TRPA1, 1:200; Abcam). Tongue sections were stained for Ki67 (rabbit anti-Ki67, 1:400; Abcam), a nuclear protein used to evaluate proliferation. After incubation in primary antibody, sections were rinsed in PBS 3 times for 10 minutes each and then incubated in the FITC-AffiniPure goat anti-rabbit secondary antibody (1:400; Jackson ImmunoResearch Laboratories) for 1 hour at room temperature. Image analysis was carried out by NIH Image J software. The area of staining was outlined and pixel density within the selected area was then measured and divided by the total area. Data were collected from 4 randomly selected sections from a minimum of 5 animals per treatment group.

ELISA measurement for cytokines. Plasma IL-6 and TNF-α were measured by using an ELISA kit from eBioscience, Inc. Plasma and fat leptin were measured by using the mouse Leptin Quantikine ELISA Kit (R&D Systems). The optical density of the standards and samples was read at 450-nm wavelength by using a Model 680 Microplate Reader (Bio-Rad Laboratories, Inc.). All samples were run in triplicate.

Statistical analysis
The statistics software SigmaPlot for Windows (version 11.0) was used to carry out all data analysis. Student’s t test or Mann–Whitney U test was used to compare mean or median for the 2 groups. Repeated-measures ANOVA with one within-subject factor (time) and one between-subject factor (treatment) followed by Holm–Sidak post hoc tests were used to compare the effect of different treatments overtime. Simple linear regression was used to examine the correlation of cytokines with changes in body weight, gnawing time, and tumor size. In the tongue model, 3 mice in the control group were euthanized at day 22 due to advanced cancer and severe cachexia. To best model the trend over time, missing values due to death were treated by using the last observation carried forward method for data analysis and figure presentation. To make sure that our results are not biased by this method, we also analyzed our data by including the missing values and found the general conclusions and statistical significance were not affected. P < 0.05 was considered statistically significant. Results are presented as mean ± SEM.

Results
NGF mRNA and protein levels were significantly elevated in oral cancer
Tissue biopsies from 14 oral SCC patients showed strong NGF immunoreactivity (Fig. 1A) whereas the normal oral epithelium from the same patients showed extremely low NGF labeling (Fig. 1B). NGF mRNA in SCC tumors was approximately 8.9 times higher than that in normal oral tissues (Fig. 1C). NGF protein concentration was also compared between HSC-3 and NOK cells. Both cell lysate and supernatant of HSC-3 cells contained much higher NGF levels than those of NOKs (89.2 ± 3.9 vs. 21.3 ± 6.6 pg/mL in lysate; 56.6 ± 3.7 vs. 20.2 ± 5.9 pg/mL in supernatant, respectively; Fig. 1D). Even greater elevation of NGF protein was found in cancer tissues collected from the mouse paw cancer model (30.3 ± 5.1 vs. 4.7 ± 2.0 pg/mg, respectively; Fig. 1E).

Anti-NGF reduced proliferation in culture and in vivo in the tongue
In HSC-3 cell culture, anti-NGF treatment significantly decreased cell proliferation at 24 (P < 0.01), 48 (P < 0.05), and 72 hours (P < 0.001) (Fig. 2A). In vivo in the tongue, treatment with the anti-NGF antibody significantly reduced tumor size at 24 (P < 0.05), 48 (P < 0.01), and 72 hours (P < 0.001) (Fig. 2B).
and 72 hours ($P < 0.01$) after treatment when compared with the corresponding control group (Fig. 2A). In the tongue tumor mouse model, anti-NGF treatment reduced tumor volume to approximately half of that in the control group ($P < 0.05$; Fig. 2C). We confirmed that the decrease in tumor volume correlated with a decrease in cancer cell proliferation through quantification of Ki-67–positive cells in total 4',6-diamidino-2-phenylindole (DAPI)-positive cells showed significantly less Ki-67 activity following anti-NGF treatment. Horizontal scale bar, 100 μm. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Anti-NGF as a Therapy in Head and Neck Cancer**

**Paw model.** Injection of anti-NGF into the mouse paw tumor reversed tactile allodynia by an average of 25% relative to that seen in both tumor + PBS ($P < 0.001$) and tumor + CL-anti-NGF groups ($P < 0.001$) throughout the observation period. Starting on postinoculation day 4, implantation of SCC cells into the right hind paws produced significant mechanical allodynia (Fig. 3A) consistent with our previous report (28). In naive mice, anti-NGF treatment did not cause a hypoalgesic effect ($P = 0.53$; Fig. 3A). Both tumor + PBS and tumor + CL-anti-NGF mice showed a decrease of the same magnitude in paw withdrawal threshold compared with naive + anti-NGF mice.

**Tongue model.** In the tongue cancer model, all mice developed visible tumor masses by postinoculation day 7. Anti-NGF treatment completely abolished the progressive increase in gnaw time caused by cancer at day 21 ($P < 0.05$), day 23 ($P < 0.01$), and day 27 ($P < 0.01$; Fig. 3B). Tumor size did not correlate positively with gnaw time ($P = 0.35$).

**Anti-NGF reduced TRPV1, TRPA1, and PAR-2 labeling**

Anti-NGF treatment led to a 26% reduction in TRPV1 labeling ($P < 0.05$), a 52% reduction in TRPA1 labeling ($P < 0.001$), and a 15% reduction in PAR-2 labeling in trigeminal ganglion cells ($P < 0.05$; Fig. 3C and D).

**Anti-NGF prevented cancer-induced weight loss**

Paw tumor groups treated with anti-NGF did not develop significant weight loss, whereas both tumor + PBS mice lost more than 5% of their baseline body mass starting at the beginning of the third week (Fig. 4A). Unexpectedly, naive mice that were treated only with anti-NGF also lost more than 5% body mass relative to their baseline at the beginning of the third week (Fig. 4A).
At days 18 and 21, anti-NGF–treated tumor mice were significantly heavier than tumor + PBS and naive + anti-NGF mice. In the tongue cancer model, by the end of the experiment, the anti-NGF–treated mice retained their original body mass, whereas the control mice lost 10% of their body mass. Significant differences in percent weight change were found at days 17 ($P < 0.05$), 21 ($P < 0.001$), 23 ($P < 0.01$), and 27 ($P < 0.01$; Fig. 4B). The transient body mass loss immediately following cancer cell inoculation (Fig. 4B) can be attributed to attenuated feeding secondary the transient tongue trauma.

**Anti-NGF modulated cytokine levels**

Mice treated with anti-NGF exhibited 50% lower plasma TNF-$\alpha$ and IL-6 (Fig. 5A and B) and 3 to 4 times higher leptin levels in plasma as well as in adipose tissue (the main source of leptin; Fig. 5C and D).

**Cytokines correlated positively with tumor size, nociception, and weight loss**

In the tongue tumor mouse models, IL-6 was positively correlated with tumor size ($R = 0.5$, $P < 0.05$), gnaw time ($R = 0.5$, $P < 0.05$), and weight loss ($R = 0.6$, $P < 0.05$; Fig. 6A–C). TNF-$\alpha$ was positively correlated with weight loss ($R = 0.8$, $P < 0.001$; Fig. 6D) but not with tumor size ($P = 0.16$) or gnaw time ($P = 0.10$). Plasma and adipose leptin concentrations were both inversely correlated with body mass loss ($R = −0.6$, $P < 0.05$; $R = −0.5$, $P < 0.05$, respectively; Fig. 6E and F) but not significantly correlated with tumor size ($P = 0.1$ and $P = 0.86$, respectively) or gnaw time ($P = 0.26$ and $P = 0.12$).

**Discussion**

Our results show that NGF affects progression of oral SCC as well as pain and cachexia associated with this cancer in patients as well as in mouse models. It does so, in part by increasing TNF-$\alpha$ and IL-6, by decreasing leptin, and by increasing expression levels of nociceptive sensory receptors. We showed that NGF production increased in the orthotopic model of human oral SCC in mice as well as in oral SCC cell culture. Moreover, NGF blockade with antibodies reduced tumor proliferation, nociception, and loss of body mass. In summary, NGF blockade (i) yielded lower levels of TNF-$\alpha$ and IL-6, (ii) upregulated leptin, and (iii) downregulated the
nociceptive receptors TRPV1, TRPA1, and PAR-2 in trigeminal ganglia.

The effect of anti-NGF on tumor proliferation varies with tumor type, tumor location, and route of administration. In this study, we evaluated the effect of anti-NGF on tumor growth in both a paw and a tongue model. For the paw model, we administered the anti-NGF locally in a manner similar to the approach used by Adriaenssens and colleagues in their study of the effect of anti-NGF in a breast cancer model (14). For the tongue model, we administered anti-NGF systemically to preclude the possibility that repeated injection into the tongue would affect feeding behavior and maintenance of body mass.

NGF is tumor promoting for a variety of cancers and anti-NGF has been shown to reduce tumor proliferation. Andriaenssens and colleagues showed that locally injected anti-NGF (12.5 μg) decreased tumor size in a breast cancer model (14). However, we found that local injection of anti-NGF (12.5 μg) in a paw model did not have a significant effect on tumor growth. In our tongue cancer model, anti-NGF significantly decreased tumor size in animals given a larger systemic dose. The suppressive effect of anti-NGF on cancer proliferation in vivo is confirmed by an anti-Ki-67 assay in the tongue cancer tissue sections and corroborated with an in vitro proliferation assay.

NGF promotes oral cancer progression in part through a mechanism that involves IL-6. We investigated whether NGF could exert its effect by modifying cytokine levels and showed that anti-NGF reduces IL-6. We also found that IL-6 was positively correlated with tumor size. This effect is supported by the clinical finding that patients with advanced oral cancer exhibit increased IL-6. IL-6 expression also correlates with poor prognosis (29, 30). Furthermore, IL-6 activates the transcription factor NF-κB and the STAT3 signal transduction pathway, which in turn regulate the expression of genes controlling cell proliferation and apoptosis in oral cancer (29). Additional mechanisms are possible. For example, NGF could also promote progression by interacting with its receptors TrkA and p75 in both an autocrine and paracrine manner.
NGF may also exert its effect on tumor progression by promoting angiogenesis. NGF has been shown to play a role in angiogenesis in ovarian and breast cancer (14, 32, 33), and NGF inhibition strongly reduces angiogenesis and tumor development in mice (14).

Previous studies showed that treatment with anti-NGF has a strong antinociceptive effect in animal models of bone cancer (16, 17). Here we present evidence that anti-NGF also exhibits a strong antinociceptive effect in mice with soft-tissue cancers. One of the pathways by which NGF induces pain and hyperalgesia entails modulation of expression and function of nociceptive receptors and sensory ion channels. For example, the TRPV1 channel is known to play a role in cancer pain (3). TRPA1 sensory channels have also been recently reported to play important roles in orofacial pain (34) and PAR-2 is involved in animal models of oral cancer pain (25). NGF upregulates TRPV1 and TRPA1 in several painful states (11, 34, 35), but a link between NGF and PAR-2 is less certain. In this study we found that NGF not only modulates TRPV1 and TRPA1 expression, but also upregulates PAR-2 expression. Most importantly, anti-NGF reduced TRPA1 by almost 50%. We infer from this finding that TRPA1 might play an important role in mediating oral cancer pain.

In agreement with previous findings (16, 17), we conclude that the antinociceptive effect of anti-NGF does not stem from a reduction in tumor proliferation alone. First, in the mouse paw model, we did not observe a significant reduction in tumor size following anti-NGF administration but a significant antinociceptive effect was found.

Second, in the tongue cancer model, no correlation was found between cancer size and gnaw time. Third, anti-NGF reduced expression of nociceptive receptors TRPV1, TRPA1, and PAR-2 in trigeminal ganglion cells. This finding shows that anti-NGF reduces pain at least in part by decreasing nociception transduction and signaling via these receptors.

Perhaps the most novel finding of our study is that NGF is associated with cancer-induced cachexia. The unexpected finding that anti-NGF given to naive mice led to reduced body mass leaves open the possibility that a basal level of NGF is necessary for maintenance of normal body mass. If basal NGF is too low, body mass is lost. However, when NGF is abnormally elevated, cachexia results. For example, in animal models of arthritis, NGF levels are elevated and anti-NGF blocks loss of body mass. These findings support the conclusion that elevated levels of NGF contribute to arthritis-induced cachexia (36). The manner by which NGF exerts its effect on body mass regulation is not entirely clear. A variety of cytokines linked to inflammation, including TNF-α, IL-6, and leptin, have been proposed to play a role in body mass regulation and cachexia (2, 22). TNF-α and IL-6 have been proposed to induce cancer cachexia through inflammation, altered metabolism, appetite suppression, and enhanced lipolysis and proteolysis (23). Elevated IL-6 and TNF-α levels have been found in cachectic cancer patients (22). Both of these cytokines correlate inversely with body mass index (BMI) in patients with gastrointestinal cancer (37, 38). Leptin is
a cytokine that is produced mainly by adipocytes (23, 39). This cytokine regulates fat mass by decreasing the level of neuropeptide Y in the hypothalamus and increasing resting energy expenditure (23). These changes result in reduced food intake. The relationship between leptin and cancer cachexia is equivocal. Serum leptin is reduced in cachectic patients with cancers of the digestive organs (37) and ovaries (40). However, leptin levels are higher than normal in breast cancer and prostate cancer patients (41, 42). In patients with oral cancer, reduced leptin levels are accompanied by decreased body mass (38, 43, 44). Our results suggest that TNF-α, IL-6, and leptin all contribute to oral cancer–induced loss of body mass. Circulating levels of these molecules can be influenced by targeting and manipulating NGF levels. Plasma levels of leptin correlate with levels in fat stores. Because plasma leptin is proportional to levels in fat stores, it is not surprising that untreated cancer mice have decreased plasma leptin because of loss of fat mass. However, the ability of adipocytes to produce leptin might have also been reduced in untreated cancer mice. NGF is known to be secreted from both murine and human adipocytes in cell culture (45, 46), and fat cells express the high and low affinity NGF receptors TrkA and P75 (45). Therefore, NGF might act directly on adipocytes to modulate leptin release. Further studies are needed to elucidate the mechanism through which NGF regulates body mass and cachexia.

Patients with advanced cancer often suffer from pain and loss of body mass. Despite the general consensus that tumor progression, pain, cachexia, and other cancer-related symptoms result from production and dysregulation of proinflammatory cytokines (1, 8), no studies have been conducted to investigate cancer-related symptoms in parallel. Such an approach might allow us to identify cellular and molecular mediators common to cancer-related symptoms for a specific tumor type.

We have identified NGF as a key endogenous factor in the panoply of molecules involved in the orchestration of cancer-related inflammation. NGF might be a molecule common to the mechanisms responsible for clinically distinctive cancer symptoms such as pain and cachexia. By suppressing proinflammatory cytokines and promoting anti-inflammatory cytokines, anti-NGF could potentially correct the altered cytokine balance observed in those who suffer from cancer. Inhibiting chronic inflammation by neutralizing NGF might be a novel and effective approach to treat pain and cachexia associated with certain cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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