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Inhibition of Fatty Acid Transport and Proliferative Activity in Tissue-isolated Human Squamous Cell Cancer Xenografts Perfused In Situ with Melatonin or Eicosapentaenoic or Conjugated Linoleic Acids

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Melatonin and eicosapentaenoic and 10t,12c-conjugated linoleic acids suppress the growth-stimulating effects of linoleic acid (LA) and its metabolism to the mitogenic agent 13-(S)-hydroxyoctadecadienoic acid (13-(S)-HODE) in established rodent tumors and human cancer xenografts. Here we compared the effects of these 3 inhibitory agents on growth and LA uptake and metabolism in human FaDu squamous cell carcinoma xenografts perfused in situ in male nude rats. Results demonstrated that these agents caused rapid inhibition of LA uptake, tumor cAMP content, 13-(S)-HODE formation, extracellular signal-regulated kinase p44/p42 (ERK 1/2) activity, mitogen-activated protein kinase kinase (MEK) activity, and [^{3}H]thymidine incorporation into tumor DNA. Melatonin's inhibitory effects were reversible with either the melatonin receptor antagonist S20928, pertussis toxin, forskolin, or 8-bromoadenosine-cAMP, suggesting that its growth-inhibitory effect occurs in vivo via a receptor-mediated, pertussis-toxin–sensitive pathway.

Abbreviations: 8-Br-cAMP, 8-bromoadenosine-cAMP; 13-(S)-HODE, 13-(S)-hydroxyoctadecadienoic acid; CLA, conjugated linoleic acid; dpm, disintegrations per minute; EPA, eicosapentaenoic acid; ERK 1/2, extracellular signal-regulated kinase p44/p42; FA, fatty acid; LA, linoleic acid; MEK, mitogen-activated protein kinase kinase; MT, melatonin receptor; PTX, pertussis toxin; TFA, total fatty acid

This year in the United States alone, more than 55,000 people will be diagnosed with squamous cell carcinoma of the head and neck, and nearly 13,000 will die from this disease.[15] This carcinoma arises from the epidermis; resembles squamous cells of the upper layers of the skin; may occur on many areas of the body, including the mucous membranes of the mouth, nose, and throat; and generally is thought to be caused by overexposure to sunlight, smoking, and excessive alcohol use. The FaDu carcinoma, named for the patient from which the tissue was removed, is classified as a grade II squamous cell carcinoma of the hypopharynx.[21] The metabolism and growth of transplantable rodent hepatomas[23,26,29] and human mammary tumors[4,22] are stimulated by linoleic acid (LA), an omega-6 fatty acid (FA), the most abundant FA in the Western diet. The enhanced growth-stimulatory response of LA results from its conversion to 13-hydroxyoctadecadienoic acid (13-[S]-HODE) in hepatoma 7288CTC[29] and MCF7 steroid-receptor–responsive and –nonresponsive human breast cancer xenografts.[27,30] The LA growth-stimulatory response is suppressed in these tumors by the circadian neurohormone melatonin,[22,30] omega-3 FAs,[4,24,27] and 10t, 12c-conjugated linoleic acid (10t, 12c-CLA).[8,31] Melatonin's mechanism of action in several tumor types involves inhibitory G protein-coupled melatonin receptors (MT_1 and MT_2), leading to modulation of adenylate cyclase, abrogation of cAMP production, suppression of LA uptake, suppression of 13-(S)-HODE release, and consequently inhibition of tumor growth and metabolism.[3,4] Although omega-3 FAs suppress the formation of proliferation-promoting eicosanoids from arachidonic acid and form mediators with decreased growth-promoting activity,[5] evidence supports the contention that tumor growth inhibition is independent of prostaglandin synthesis.[30] Investigations in vitro using rodent and human cell lines[7,18] have demonstrated the presence of G-protein-coupled plasma-membrane free fatty acid receptors, specific for various FAs, including 10t, 12c-CLA. Other investigators have shown the growth-inhibitory effects of 10t, 12c-CLA in vitro on several human tumor cell lines.[1,17,34] Recent in vivo studies suggest that the growth-inhibitory effects of melatonin, omega-6 FAs, and 10t, 12c-CLA are dependent on a specific signal transduction pathway that is common to both rodent[28] and human tumors.[30] It is unclear whether these agents might also be involved independently to activate a common early signal.

In this study, xenotransplants of FaDu, an epithelial cell line derived from human pharyngeal squamous cell carcinoma,[21] are grown as 'tissue-isolated' tumors in male nude rats. We used a novel technique we developed several years ago[9,33] to perfuse tumors with blood from donor animals that was either deplete (control) or replete (experimental) with melatonin, eicosapentaenoic acid (EPA, an omega-3 FA), or 10t, 12c-CLA. We tested the hypothesis that melatonin, EPA, and 10t, 12c-CLA each would inhibit tumor cAMP levels, FA uptake, LA metabolism to 13-HODE,

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extracellular signal-regulated kinase p44/p42 (ERK 1/2) activity, mitogen-activated protein kinase kinase (MEK) activity, and [^{3}H] thymidine incorporation into tumor DNA in human FaDu xenografts. In the case of melatonin, we further postulated that these inhibitions would be reversed by addition of the melatonin receptor antagonist S20928, 8-bromoadenosine-cAMP (8-Br-cAMP), or pertussis toxin (PTX) to the melatonin-containing arterial blood perfusate. Recent in vivo studies suggest that the tumor-growth–inhibitory effects of melatonin, omega-6 FAs, and 10t, 12c-CLA are mediated through specific inhibitory G protein-coupled signal transduction pathways.

Materials and Methods

Animals, housing conditions, and diet. Adult male, homozygous, athymic, inbred nude rats (Hsd:RH-*Foxn1^{rnu}*) were purchased from Harlan (Indianapolis, IN). Adult male Buffalo rats (BUF/CrCrl; 250 to 300 g), which provided donor blood for perfusions, were purchased from Charles River Laboratories (Wilmington, MA). Both specific-pathogen-free strains were maintained in environmentally controlled rooms (23 °C, 45% to 50% humidity) in microisolation caging (Thoren Caging Systems, Hazleton, PA) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and in accordance with the *Guide for the Care and Use of Laboratory Animals*.[19] This investigation was approved by the institutional animal care and use committee. To ensure that all study animals remained uninfected with bacterial or viral agents, serum samples from sentinel rats were tested by enzyme-linked immunosorbent assay (Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan H1 virus, Theiler murine encephalomyelitis virus, reovirus, *Myocoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, Hantaan virus, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, parvovirus NS1, rat parvoviruses, and rat murin virus; Comprehensive Health Monitoring Program, Charles River Laboratories, Kingston, NY) throughout the course of this study. Lighting was diurnal, 12:12-h light:dark (lights on, 0600; 300 lux); animals were completely free from any ocular light at night exposure. Animals were provided free access to water and an essential fatty acid-replete diet (Prolab RMH 1000, Agway, Syracuse, NY) assayed as described previously.[8,25,26,31]

Surgical procedures. FaDu human carcinoma xenografts were implanted in male athymic nude rats (Hsd:RH-*Fox1^{rnu}*) and grew as tissue-isolated tumors as previously described,[23,25] with a single arterial and venous connection to the host. The FaDu tumors were verified histopathologically to be like the FaDu cell line of grade II human hypopharyngeal squamous cell carcinoma.[21] Latency to onset of tumor growth and estimated tumor weights were measured and recorded.[25]

Arterial and venous measurements in FaDu human cancer xenografts during perfusion in situ. When estimated tumor weights were approximately 4.5 to 6.0 g, tumors were prepared for in situ perfusion. Experiments were conducted between 0700 and 1000 after a normal nocturnal feeding period. Animal preparation, including anesthesia administration, heparin-treatment, surgical preparation of the tumor, and blood sample collection, has been described previously.[4,8,26,27,30,32] The tumor perfusions lasted for either 60 or 150 min. Arterial and venous samples for total fatty acid (TFA, sum of myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidonic acids) and LA uptake, 13-(S)-HODE, glucose and lactic acid determinations, blood gas analysis, and hematocrit were collected at 30-min intervals. Donor blood was supplemented with 1 nM melatonin, 0.06 to 1.00 mM EPA (Sigma Scientific, St Louis, MO), or 0.11 mM 10t, 12c-CLA (Cayman Chemical, Ann Arbor, MI), in a manner as described previously.[23,24,27,30] Analysis of the melatonin revealed 100% purity; assay of EPA and the 10t, 12c-CLA isomer by gas chromatography indicated greater than 99% purity, in agreement with the chemical supplier's specifications. Depending on the melatonin experiment, the donor arterial blood also was supplemented with PTX (0.5 µg/ml plasma; an agent that prevents dissociation of the inhibitory $G_i\alpha\beta\gamma$ heterotrimer), 10 µM 8-Br-cAMP (Sigma Scientific), or 1 nM melatonin receptor (MT1 and MT2) antagonist S20928 (Servier, Courbevoie Cedex, France). At 20 min before the end of the perfusion, [methyl-^{3}H]thymidine (20 µl [2 µCi]/g estimated tumor weight; New England Nuclear, Boston, MA) was injected into a side port of the arterial catheter leading to the tumor and made 1 passage through the tumor. All radioactivity incorporated in tumor DNA was measured by liquid scintillation by using an internal standard and is reported here as disintegrations per minute (dpm) per microgram of tumor DNA. Tumor DNA content was measured fluorometrically.[4,26,33] At the conclusion of each perfusion, FaDu tumors were freeze-clamped by using a liquid nitrogen-chilled aluminum clamp and stored at –80 °C for determination of cAMP content, [^{3}H]thymidine incorporation, and expression of total and phosphorylated ERK (ERK1 and ERK2) and MEK (MEK1 and MEK2), as described previously.[2,8,26,28]

Fatty acid extraction and analysis, and 13-HODE, glucose, and lactic acid determination. Plasma TFAs were extracted from 0.1-ml arterial and venous samples after addition of heptadecanoic acid internal standard. Arterial–venous difference measurements were calculated as rates of FA uptake or release and were expressed as micrograms per minute per gram of tumor tissue. 13-(S)-HODE was separated by means of high-performance liquid chromatography from 0.2 ml plasma and measured at 234 nm.[4,8,10,25] Arterial and venous glucose and lactic acid concentrations for arterial–venous difference measurements were determined directly from plasma samples by using a spectrophotometer (MIRA, Roche, Roswell, GA), as previously described.[20,33]

Statistical analysis. All data are presented as mean ± 1 standard deviation and were compared by using 1-way analysis of variance followed by Student–Neuman–Keul post-hoc tests. Differences among the group means were considered statistically different when the P value was less than 0.05.

Results

The data presented in Table 1 reveal marked differences in the rates of FA uptake and associated downstream events by FaDu human hypopharyngeal cancer xenografts perfused in situ with either melatonin, EPA, or 10t, 12c-CLA. Perfusates containing arterial blood supplemented with 1 nM melatonin attenuated tumor cAMP levels by more than 75%, inhibited TFA and LA uptake, abolished 13-(S)-HODE production, and brought about a 50% reduction in tumor [^{3}H]thymidine incorporation and DNA content, compared with controls. Tumor perfusions with 0.37 mM EPA-supplemented blood brought about a nearly 60% decrease in cAMP levels and caused TFA and LA uptake rates to be reduced 100%, compared with those of controls, with corresponding inhibition of tumor 13-(S)-HODE release. [^{3}H]thymidine incorporation into tumor DNA and DNA content as compared with

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Table 1. Effects of perfusion (150 min) of tissue-isolated FaDu human squamous cell xenografts with melatonin (1 nM), EPA (0.37 mM), 10t, 12c CLA (0.11 mM), or vehicle only (control) on tumor cAMP levels, TFA and LA uptake, 13-HODE production, [^{3}H]-thymidine incorporation, and DNA content

Treatment (n = 3/group)	cAMP (nmol/g tissue)	TFA uptake (μg/min/g)	LA uptake (μg/min/g)	13-HODE venous release (ng/min/g)	[^{3}H]thymidine incorporation (dpm/μg DNA)	DNA content (mg/g)
Fed control	0.958 + 0.190	6.96 + 2.90	1.69 + 0.89	18.89 + 5.11	52.9 + 4.7	3.9 + 0.2
Fed → melatonin	0.233 + 0.050[a]	(0.34 + 0.96)[a,b]	(0.14 + 0.16)[a,b]	ND	26.5 + 4.3[a]	1.9 + 0.2
Fed → EPA	0.404 + 0.029[a]	(0.05 + 0.12)[a,b]	(0.02 + 0.02)[a]	ND	22.5 + 5.1[a]	2.2 + 0.2
Fed → 10t, 12c CLA	0.409 + 0.026[a]	(0.01 + 0.16)[a,b]	(0.01 + 0.09)[a,b]	ND	21.5 + 10.6[a]	2.1 + 0.2

ND, none detected.
[a]$P < 0.05$ versus respective controls.
[b]Parentheses indicate TFA release by the tumor.

control values were attenuated by approximately 58% and 43%, respectively. Perfusion of tumors with donor blood supplemented with 0.11 mM 10t, 12c-CLA lowered cAMP levels by almost 60% and, in turn, caused TFA and LA uptake and 13-(S)-HODE release to be suppressed completely, as compared with controls, with corresponding attenuation of 59% and 45% in [^{3}H]thymidine incorporation into tumor DNA and DNA content, respectively. Tumor uptake of EPA was 0.45 ± 0.15 μg/min/g tissue, representing 15.3% ± 3.4% of that supplied. In comparison, tumor uptake of 10t, 12c-CLA was 0.38 ± 0.08 μg/min/g tissue, equivalent to 50.7% ± 2.2% of that supplied to the tumor.

The rates of TFA and LA uptake (Figure 1 A) and 13-(S)-HODE release (Figure 1 B) diminished to zero as the plasma concentration of EPA increased. In light of the decrease in TFA uptake, the calculated K_i value in the arterial blood was approximately 50 μM. Tumor [^{3}H]thymidine incorporation (Figure 1 B) and DNA content decreased from 52.9 ± 4.7 dpm/μg DNA and 3.9 ± 0.2 mg DNA/g tumor, respectively, to a nadir of 22.5 ± 5.1 dpm/μg DNA and 2.2 ± 0.2 μg DNA/g tumor at about 0.37 mM EPA. Uptake of EPA was dose-dependent and continued at concentrations as high as 1 mM, even though TFA uptake was abolished completely (inset, Figure 1 A).

Arterial and venous determinations for pH, pO_2, and pCO_2, in control, MLT, EPA, and 10t, 12c-CLA treatment groups did not vary significantly during the course of these perfusions and are represented here as the mean values of the combined measurements; therefore, mean values were 7.44 ± 0.02, 155 ± 18, and 32 ± 3 in arterial blood and 7.31 ± 0.03, 33 ± 4, and 57 ± 4 in the tumor venous blood, respectively. Rates of arterial and venous bloodflow were 0.128 ± 0.003 and 0.123 ± 0.003 ml/min, respectively, and did not vary among groups. Arterial and venous hematocrit values revealed only minor difference between each other, and this difference remained consistent and similar between control and treatment groups; there was slight hemoconcentration (2.0% ± 0.01%) as blood passed through the tumor. The presence of the melatonin, EPA, or 10t, 12c-CLA isomer had no effect on tumor O_2 consumption, CO_2 production, bloodflow, or hematocrit, in good agreement with what has been reported earlier for the perfused tissue-isolated hepatoma[4,8,11,23-29] and human breast tumor models[4,30] and the inguinal fat depot.[28]

Figure 2 shows the kinetics of changes from steady-state rates of TFA and LA uptake and 13-(S)-HODE release by the tumor in the absence of melatonin to the inhibited steady-state, after addition of melatonin to the arterial blood perfusate. Within 20 min after addition of melatonin, with time allowed for circulation through the tumor, the onset of the inhibitory effect of melatonin on FA uptake and 13-HODE release occurred rapidly. Perfusion with 1 nM melatonin significantly ($P < 0.001$) reduced these measures to about 75% of control levels. The inhibitory effect of 1 nM melatonin was completely reversed by addition of either the melatonin antagonist S20928 (1.038 ± 0.149 nmol/g; Figure 2 A), PTX (0.920 ± 0.187 nmol/g; Figure 2 B), or 8-Br-cAMP (1.043 ± 0.411 nmol/g; Figure 2 C), or forskolin (10^{-6} M, data not shown). Interestingly, addition of 13-(S)-HODE to the 1 nM melatonin-supplemented blood perfusate raised cAMP levels more than 2-fold, compared with those of controls, whereas uptake of TFA and LA remained completely abrogated.

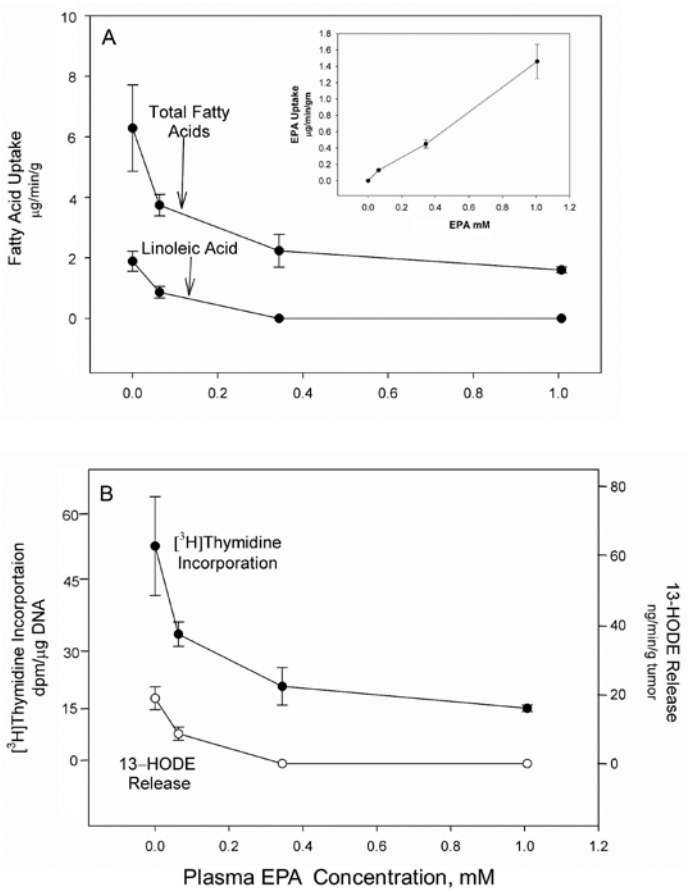

Figure 1. The effects of increasing concentrations of arterial blood plasma of EPA on (A) TFA and LA uptakes, (B) 13-(S)-HODE release in tumor venous blood, and DNA [^{3}H]thymidine incorporation in FaDu tumor xenografts perfused for 60 min in situ. Each point represents the mean value (± 1 standard deviation) from 3 tumors. Mean tumor weight was 5.31 ± 0.59 g. The inset shows the rate of EPA uptake in the 4 experimental groups.

All tumors in this investigation used glucose and produced lactic acid. Regardless of the treatment regimen, there were no

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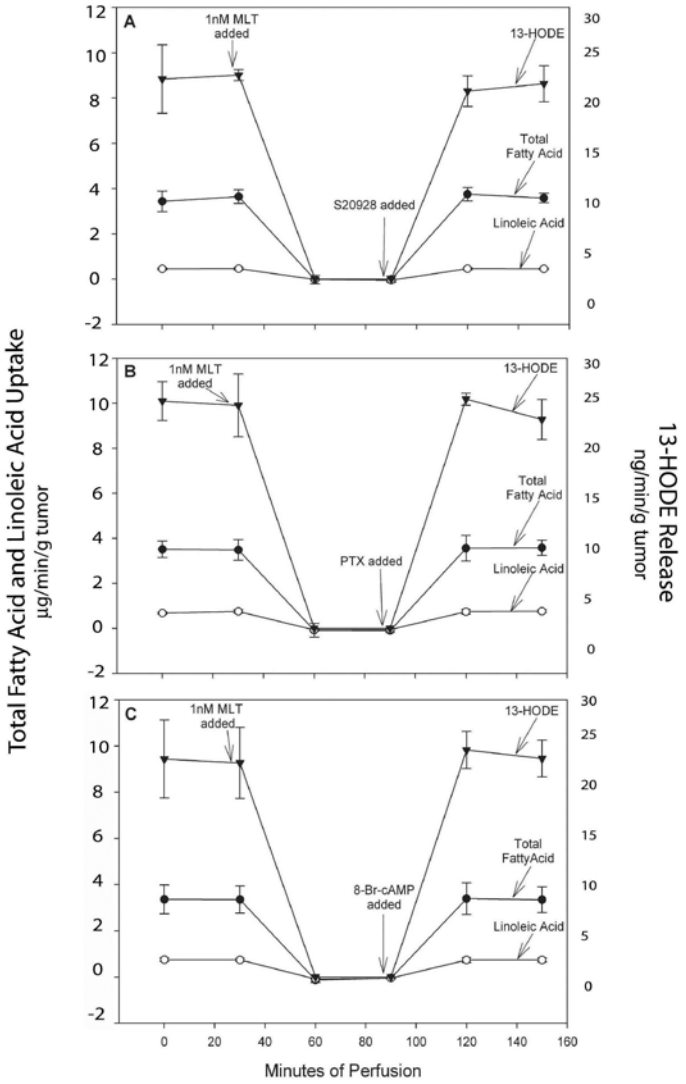

Figure 2. Kinetic changes in TFA and LA uptakes and 13-(S)-HODE release in FaDu human squamous cell cacinomas perfused for 150 min in situ after consecutive supplementations to the arterial blood perfusate of melatonin followed by either antagonist S20928 (A), PTX (B), or 8-Br-cAMP (C). Melatonin (MLT) was added at 6 min after the 30-min sample collections; antagonist S20928, PTX, or 8-Br-cAMP was added 4 min prior to the 90-min collections. Each value depicted here represents the mean (± 1 standard deviation) from 3 tumors. Mean tumor weight was 6.0 ± 0.9 g.

significant alterations in either arterial or venous glucose or lactic acid concentrations, glucose utilization, or lactic acid production. The mean arterial glucose concentration was 6.72 ± 0.99 mM (n = 54). Mean glucose utilization rates were 61.96 ± 8.58 nmol/min/g tumor wet weight and were directly proportional to the rate at which glucose was supplied to the tumor. The relationship between glucose supply to the tumor and glucose utilization was described by linear regression analysis. A least-squares fit to the data points (n = 54) reveals high correlation ($r = 0.967$, $P < 10^{-6}$) and the line y = 0.35x + 1.338 (Figure 3). Depending on the supply rate, the FaDu tumors utilized between 31% and 37% (35.7% ± 1.6%) of the glucose supplied to the tumor, demonstrating that these tumors in vivo have a large capacity for utilizing glucose. Similar relationships were described previously by Gullino and colleagues[13] and

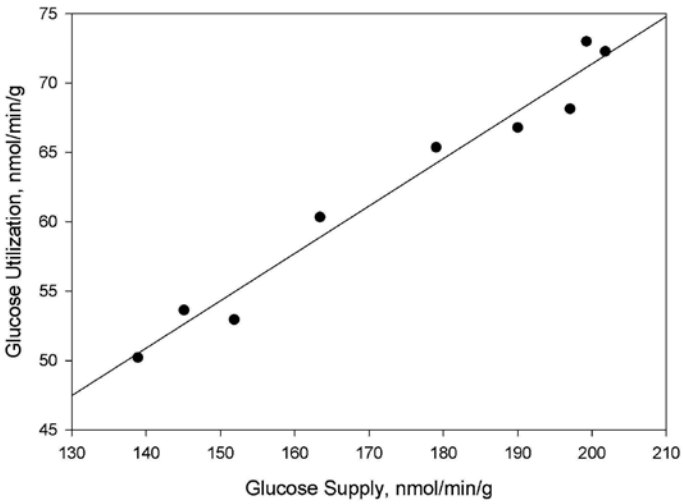

Figure 3. Relationship between the rate of glucose supply and the rate of glucose utilization in FaDu tumors in vivo. Each point represents the mean of 6 time point determinations of a single perfusion. The line is a least-squares fit to the data points.

Sauer and colleagues[33] for glucose utilization and supply in rodent tumors in vivo. In addition, the mean arterial lactic acid concentration was 1.160 ± 0.108 mM, whereas tumor production of lactate was 115.89 ± 20.24 nmol/min/g wet weight.

Figure 4 depicts Western blots of phosphorylated (upper) and total (lower) ERK (ERK1, p42; ERK2, p44; Figure 4A) and MEK (p45, Figure 4 B) in FaDu human squamous cell tumors freeze-clamped after perfusions in situ with arterial blood containing the various TFA inhibitory agents. Expression of the phosphorylated, active forms of ERK 1/2 and MEK were almost completely suppressed in tumors perfused with arterial blood containing 1nM melatonin, 0.37 mM EPA, or 0.11 mM 10t, 12c-CLA isomer. The decreases in ERK 1/2 and MEK expression were restored after addition of either PTX, forskolin, 8-Br-cAMP, or 13-(S)-HODE (data not shown) to the blood containing melatonin.

Discussion

The purpose of this study was to examine rapid changes in FA transport and proliferative activity in perfused tissue-isolated human squamous cell cancer xenografts in situ following the addition of growth-inhibitory agents to the whole-blood perfusates. Using a unique tumor perfusion system developed in our laboratory,[8,11] we examined the effects of the circadian neurohormone melatonin, the omega-3 FA EPA, and 10t, 12c- CLA on inhibition of TFA uptake, tumor metabolism, and proliferation. Previous studies from our laboratory demonstrated that the growth rate in vivo and [^{3}H]thymidine incorporation in hepatoma 7288CTC[2,11,23,24,26,29,32] and human breast cancer xenografts[2,8,27,30,31] perfused in situ were dependent on and controlled by the rate of formation of 13-(S)-HODE from LA. When dietary concentrations and animal intake of LA increase, blood concentrations rise accordingly. Because of higher cAMP levels, there is increased entry of LA into tumor and growth rates increase concomitantly. The enzyme 15-lipoxygenase, located within tumor cells, converts LA to the potent mitogenic metabolite 13-(S)-HODE, thus stimulating tumor growth and metabolism via the epidermal growth factor receptor–mitogen-activated protein kinase pathway. Subsequent investigations[4,8,24,26,27,29,30,31] demonstrated that melatonin,

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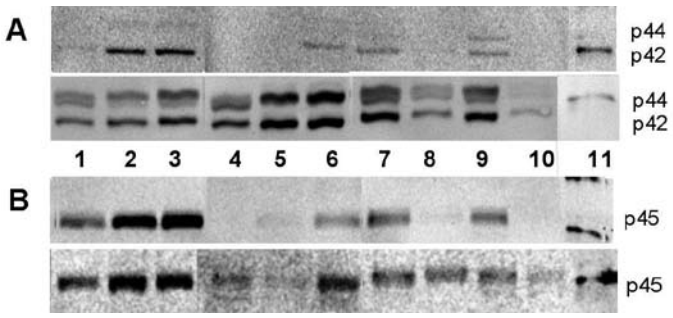

Figure 4. Western blot analyses for the expression of total (lower panel) and phosphorylated (upper panel) forms of (A) ERK1and 2 (p42 and p44) and (B) MEK (p45) in FaDu human squamous cell carcinomas xenographs perfused in situ. Each lane represents data from 1 tumor perfused for 60 min. Perfusions were: lanes 1 through 3, controls (no inhibitory agent); lanes 4 through 6, 1 nM melatonin; lanes 7 and 8, 0.37 ± 0.05 mM EPA; lanes 9 and 10, 0.11 ± 0.004 mM 10t, 12c-CLA; lane 11, molecular weight standards.

omega-3 FAs, and conjugated linoleic acid suppress uptake of LA by these tumor tissues. The mechanism of action occurs via a receptor-mediated G inhibitory protein, which suppresses adenylyl cyclase activation and decreases cAMP levels. This effect, in turn, leads to decreased TFA and LA uptake; suppression of 13-HODE formation; associated decreased phosphorylated ERK 1/2 and MEK activation; and resultant inhibition of tumor [^{3}H] thymidine incorporation and growth. Interestingly, similar effects were noted subsequently in perfused inguinal fat pads[11,30] and rodent hindlimb skeletal muscle[20]—2 organs that play a pivotal role in cancer cachexia and obesity-related diseases.

Increased consumption of diets rich in LA, such as found in the Western diet, increase tumorigenesis and growth in transplanted human cancer xenografts.[8,22,24,27,30,31] Conversely, consumption of diets rich in melatonin[3,4] and omega-3 FAs, including EPA, or 10t, 12c-CLA may influence or inhibit tumorigenesis and growth.[8,22,24,27,30,31] In addition, disruptions in normal circadian hormone concentrations[3] or dietary alterations[30] can effect changes in the progression of the disease state of tumors. Although the regulating factors for the metabolic and physiologic changes associated with the diseased state of FaDu human squamous cell carcinoma are still somewhat unclear, it now appears that a similar mechanism underlying the tumor cell growth and proliferation, as shown with other tumors[4,8,24,27,29,30,31], may be involved with this human tumor. In the case of EPA, inhibition of tumor LA uptake and growth is dose-dependent with increasing blood concentrations of EPA. In addition, although the uptake of all other saturated and mono- and polyunsaturated FAs was completely suppressed, uptake of EPA continued, suggesting that uptake of omega-3 FAs occurs independently of other FAs. Previous studies using other rodent and human tumors[2,8,30,31] have shown similar dose-dependent relationships with melatonin, EPA, and 10t, 12c-CLA.

The important advantage of the model system we used is the ability to investigate healthy tissue directly, without long periods of anoxia or nutrient deprivation. Because the actual surgical procedure caused minimal disturbance to the tissue and because rat blood hemoglobin was the natural oxygen carrier, arterial and venous whole-blood pH, pO_2, and pCO_2 and blood nutrients remained within physiologic limits and constant throughout the course of each perfusion, even after the addition of growth-inhibitory agents.

We believe that the effects of melatonin, EPA, and 10t, 12c-CLA are mediated by either a melatonin receptor or an inhibitory G protein-coupled free fatty acid receptor[35] and that a specific ligand structure is required. However, of the 3 compounds used here in the perfused FaDu squamous cell carcinoma xenografts, only the actions of melatonin are mediated by well-documented inhibitory G protein-coupled melatonin receptors, MT_1 and MT_2. The inhibitory effect of melatonin on intratumor cAMP, FA transport, 13-(S)-HODE formation, activity of phosphorylated ERK 1/2 and MEK, and [^{3}H]thymidine incorporation in FaDu tumors was reversed by compound S20928, a specific MT_1 and MT_2 antagonist; PTX, an agent that inhibits the dissociation of the inhibitory $G_i\alpha\beta\gamma$ heterotrimer; 8-Br-cAMP; and forskolin, a known adenylate cyclase activator that elevates intracellular cAMP.

The traditional school of thought[36] supports cell membrane diffusion of FAs, which occurs as a result of their lipophilic nature. However, a growing body of more recent evidence suggests that transport of FAs occurs via a specific carrier-mediated pathway.[12,16] Indeed, the fatty acid transport 1 gene is highly expressed in tissues with high rates of metabolism, including tumor tissues.[38-40] The evidence presented here provides strong support that melatonin, EPA, and 10t, 12c-CLA in the arterial blood can attenuate rapidly a signal transduction pathway for cell proliferation and metabolism in human FaDu squamous cell carcinoma xenografts and argues strongly for the involvement of such a facilitated transport system in FaDu human squamous cell carcinoma. The inhibitory agents used in this study bind to either a melatonin receptor or a putative G protein-coupled free fatty acid receptor; 2 possible inhibitory candidates are GPR40 and GPR120, which are expressed in several rodent and human tumors.[6,14,35] Sauer and Blask[30] recently reported that MT_1 and MT_2 mRNA transcripts are expressed in MCF7 (steroid-receptor–positive and –negative) human breast tumor xenografts. The tumor-suppressive effects of melatonin that are completely blocked by the nonselective melatonin receptor antagonist S20928 argue convincingly that the mechanism involved is a melatonin receptor-mediated process. Studies are now underway to delineate which melatonin receptor may be involved in FaDu human squamous cell carcinoma, as has been determined for hepatoma 7288CTC[4] and the MCF7 human breast tumors.[2]

In recent years, increasing attention has focused on melatonin, EPA, and CLAs and their potential roles in cancer prevention.[3] The uptake of LA and its conversion to 13-(S)-HODE is critical for tumor cell growth and metabolism. Here we detail a unique interaction between 2 environmental factors that regulate metabolism and growth in human squamous cell carcinoma. The circadian neurohormone melatonin and dietary fats, including the omega-3 FA EPA and 10t, 12c-CLA, are involved integrally in overall TFA uptake and metabolism by this tumor. To our knowledge, these findings are the first in vivo evidence in human hypopharyngeal squamous cell carcinomas that these agents attenuate cell proliferation by inhibiting the rate of LA uptake. In the case of melatonin, this attenuation occurs via a MT_1- or MT_2-mediated, PTX-sensitive pathway that suppresses adenylyl cyclase activity and ERK activity. Additional studies are warranted to examine potential long-term, beneficial effects of dietary supplementation with melatonin, EPA, and 10t, 12c-CLA and to further elucidate the mechanism(s) surrounding their anticancer properties.

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