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LNCaP Prostate Cancer Growth *In Vivo*: Oncostatic Effects of Melatonin as Compared to Hypoxia and Reoxygenation

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1. Introduction

Oxygen (O_2) is often thought of as a double-edged sword: all forms of life actually need O_2 for survival as the terminal acceptor of electrons in oxidative phosphorylation, but excess O_2 might increase formation of reactive O_2 species (ROS). Whereas on one hand ROS trigger uncontrolled burst of free radicals that lead to potentially lethal injury, on the other hand they act as messengers that elicit cell protection and improve survival through a variety of mechanisms. Although one can easily expect a link of O_2 with tumor growth and metastatic potential, there is no univocal role for O_2 in cancer.

1.1 Hypoxia in cancer

Lack of O_2, or hypoxia, has been extensively studied in cancer because the growth of solid tumors requires a local vascular network that supplies O_2 and nutrients to tumor cells. In the classical view, when cell proliferation exceeds angiogenesis, the vasculature might become unable to sustain the O_2 needs of tumor cells, which therefore have to cope with an environment chronically deficient in O_2 as a result of diffusion-limited O_2 supply (Vaupel, 2004). This triggers various mechanisms, most of which are mediated by over-expression of the hypoxia-inducible factor-1 (HIF-1α). HIF-1α stimulates a variety of mechanisms aimed at survival of hypoxic tissue, for example the angiogenic switch, which provides growth factors for the development of circulation to feed the growing tumor, as well as anti-apoptotic and cell cycle factors. Therefore, tumor hypoxia emerges as a major contributor to the malignant phenotype (Hockel *et al.*, 2001) and a cause of resistance to radiation therapy (Brown *et al.*, 1998).

There are several instances whereby the O_2 supply to tissues is altered. The term "hypoxia" is sometimes improperly attributed to any of them, but there are important differences with dramatically different phenotypes, as explained in Table 1.

* These Authors contributed equally to the work

Name	*Relative experimental situation*	*Correlated pathologies*
Chronic hypoxia	Prolonged O_2 supply/demand unbalance without interruption, typical of decreased tissue perfusion for altered geometry of O_2 diffusion from capillary to cell	Chronic obstructive pulmonary disease, congenital heart disease, cancer-derived anemia, blood O_2 carrying failure, CO poisoning, high altitude
CH with repeated reoxygenation	Animals housed in hypoxic chambers that are opened for cleaning and animal feeding	Some cases of immature capillary network with pulsing perfusion changes
Intermittent hypoxia	Repetitive hypoxic events, typical of immature capillary network with pulsing perfusion changes	Obstructive sleep apnea, sickle cell anemia crises, asthma, immature capillary network with pulsing perfusion changes

Table 1. Different types of hypoxia and examples of correlated pathologies.

1.2 Prostate cancer

Prostate cancer is the most common neoplasia and the second most frequent cause of male cancer death in the developed world and in many Western countries (Hsing *et al.*, 2000). A malignant tumor derived from the interaction of genetic and environmental factors, potentially curative treatment options are available for management of early localized disease, whereas palliative hormonal therapy in the form of medical or surgical castration is the mainstay of treatment for patients with advanced prostate cancer. Approximately 80% of castrated patients will suffer from a relapse of the disease within 2 years, with progression of the tumor from a hormone-dependent to a hormone-independent stage (Wilding, 1995), which is associated with unfavorable prognosis.

In addition, prostate cancer may also represent an useful workbench to investigate the relationship between hypoxia and cancer, because HIF-1α is over-expressed compared with normal prostate epithelium (Zhong *et al.*, 1999) and its up-regulation is recognized as an early event in carcinogenesis (Zhong *et al.*, 2004). Furthermore, androgens and androgen receptors modulate HIF-1α levels (Kimbro *et al.*, 2006), and hypoxia increases androgen receptor activity in LNCaP cells in *vitro* via HIF-1α (Park *et al.*, 2006).

1.3 Melatonin and prostate cancer

Melatonin (N-acetyl-5-methoxytryptamine, **Figure 1**), a neurohormone synthesized during night time in the pineal gland, mediates many physiological, endocrinological and behavioral processes, including the well-known biorhythmic regulation of organism physiology, through its action on the biological clock at the hypothalamic suprachiasmatic nucleus (Tamarkin *et al.*, 1985). Melatonin increases sleepiness, decreases core temperature, and increases peripheral temperature in humans (Brzezinski, 1997; Burgess *et al.*, 2001; Lewy *et al.*, 1996). The melatonin's regulatory roles are mediated through high affinity G protein–coupled receptors that reside primarily in the eye, kidney, gastrointestinal tract, blood

vessels, and brain (Beyer *et al.*, 1998). This suggests some significant actions of melatonin on the cell biology of these target tissues outside the central nervous system as well (Cardinali *et al.*, 1997; Pang *et al.*, 1993). In addition, melatonin is known to exhibit antioxidant properties against the deleterious effects of reactive oxygen and nitrogen species (ROS and RNS, respectively) that are independent of its many receptor-mediated effects (Korkmaz *et al.*, 2009; Ochoa *et al.*, 2011). Melatonin has been reported to scavenge hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), nitric oxide (NO•), peroxynitrite anion ($ONOO^-$), hypochlorous acid (HOCl), singlet molecular oxygen [$O_2(^1\Delta_g)$] and superoxide anion ($O_2^{\bullet -}$) (Allegra *et al.*, 2003; Reiter, 1998; Reiter *et al.*, 2001; Tan *et al.*, 2000). Melatonin has also been shown to possess genomic actions, through regulation of the expression of several genes including glutathione peroxidase, superoxide dismutase, and catalase, both under physiological conditions and under conditions of elevated oxidative stress (Allegra *et al.*, 2003; Kotler *et al.*, 1998).

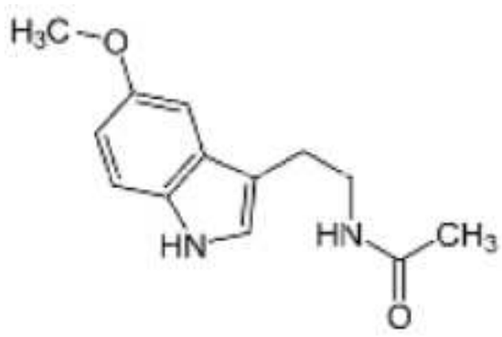


Fig. 1. Structure of melatonin, MW 232.2 Da.

A multitude of literature reports have documented a direct modulatory effect of melatonin on benign and malignant cell proliferation and an anti-tumor effect was reported both in vitro and in vivo in human tumors of the reproductive tissues (Cos *et al.*, 1998; Shiu *et al.*, 1999). Among these, a significant role of melatonin, by itself and by interaction with sex steroids, has been found in the pathobiology of prostate cancer and benign prostatic hyperplasia (Laudon *et al.*, 1996; Lupowitz *et al.*, 1999). Of the various experimental prostate cancer models, hormone (androgen)-sensitive and hormone (androgen)-insensitive metastatic human prostate cancer cell lines are widely used for the experimental evaluation of pharmacological agents with therapeutic potential for the disease (Cho *et al.*, 2011). In particular, the growth of the androgen-independent but androgen-sensitive (responsive) human LNCaP prostate cancer cells has been demonstrated to be inhibited by the pineal gland indoleamine hormone both in vitro and in vivo in a nude mice xenograft model (Siu *et al.*, 2002; Xi *et al.*, 2000). The antiproliferative action of melatonin seems to be mediated in part by means of MT1 receptor activation and partly by means of attenuation of dihydrotestosterone-induced calcium influx model (Xi *et al.*, 2000).

1.4 Solid lipid nanoparticles and cryopass laser therapy

Cytostatic and antitumoral drugs activity is often impaired by low plasma solubility, poor systemic absorbance, rapid metabolism, non-specific tissue distribution and toxic effects. The search for advanced methods to deliver these molecules to target tissues and to overcome the failure shown even with very active drugs is therefore mandatory.

Solid-lipid nanoparticles (SLN) is a technology able to produce sub-micrometric lipidic particles characterized by an average diameter <500 nm, with a narrow size distribution and a spherical shape. The several advantages of this technology are listed in Table 2 (Mehnert *et al.*, 2001). SLN formulation of antitumor drugs is predicted to be advantageous with respect to other formulations because it allows greater uptake into the malignant cell, with consequent intracellular accumulation and higher efficacy, still keeping the level of free circulating drug as low as possible, thereby preventing non-specific unwanted side effects.

Main advantages of SLN formulation for drugs delivery.
To control and direct the release of the drug against a specific target organ
To modify favorably drugs pharmacokinetics
To target directly to lymph bypassing the entero-hepatic circulation following oral administration
To obtain a stealth formulation that avoids recognition by the reticuloendothelial system
To allow oral administration of drugs poorly soluble and not easily adsorbed in the gastrointestinal tract
To overcome the blood-brain barrier
To permit administration routes as transdermal or eye topical

Table 2. Main advantages to administer drugs as solid-lipid nanoparticles (SLN).

Criopass therapy, is a procedure used to actively deliver drugs across the dermal barrier. It is based on topical application of a frozen drug emulsion in 1.5% hydroxymethylcellulose by means of a laser source that gives energy to penetrate the dermal barrier and deliver the active principle to the target area. The low energy photon flux generated by a laser beam hit the drug molecules frozen in the crystal lattice, exciting the electrons in the outer orbital. The drug molecules, when melt at the ice-skin interface, release the accumulated potential energy transforming into kinetic energy, which speeds up the passage of the drug across the skin membrane and allows to reach the target area. The last step consists in a laser scan on the area of drug application to optimize the adsorption through the skin and facilitate the drug to reach the desired site of activity. This treatment is particularly advantageous for treatment of bones cartilage, producing significant drug accumulation in a tissue difficult to be reached by traditional administration techniques. The main advantages of this non-invasive and painless treatment are speed of absorption (15-20 s), high capacity of penetration (6 cm depth), suitability for polar and non-polar molecules delivery, improved drug bioavailability and high specificity.

1.5 Aims

Solid tumors contain underperfused regions where hypoxia might induce adaptation and cell proliferation. Often, this response is mediated by HIF-1α over-expression in hypoxic

cells. We demonstrated that systemic chronic *in vivo* hypoxia promotes prostate cancer growth regardless of HIF-1α expression level and neovascularization. We have also assessed that altering HIF-1α expression by use of non-pharmacological agents (intermittent hypoxia with reoxygenation) alter the phenotype of tumor growth. These observations suggest an important role for hypoxia dependent pathways that do not involve HIF-1α, and for the pharmacological treatments able to modulate these pathways.

Pharmacologic concentrations of melatonin inhibit *in vitro* expression of HIF-1α protein under both normoxic and hypoxic conditions in DU145, PC-3, and LNCaP prostate cancer cells (Park *et al.*, 2009). This effect, perhaps a result of the antioxidant activity of melatonin against ROS induced by hypoxia, and the subsequent suppression of HIF-1α transcriptional activity decreases VEGF expression in HCT116 human colon cancer cell line (Park *et al.*, 2010). Therefore, the main aim of this preliminary study was to focus into the role of melatonin as a therapeutic/adjuvant agent that reduces tumor growth in vivo as a proof-of-concept for further studies assessing melatonin interference with the hypoxia signaling paths. To this purpose, we used the *in-vivo* model of nude mice xenograft with human LNCaP prostate cancer cells under a variety of conditions spanning from chronic hypoxia with/out reoxygenation, and compared different routes of melatonin administration.

2. Methods

Cells. LNCaP cells (80-90% confluence) were maintained in RPMI-1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum and L-glutamine, and cultured in 5% CO_2. To ensure that LNCaP cells were not injured when passing through G26 needles during xenografts, we verified that their vitality did not decreased by more 2-3% per each passage. To obtain positive controls for HIF-1α immunostaining, LNCaP cells were incubated for 24 h in the presence of 100 μM $CoCl_2$, washed, fixed in formalin and stained as described below.

Mice. Seven-week old Foxn1$^{nu/nu}$ mice (Harlan, n=46), weighing 25-30 g at the entry into the study, were cared in accordance to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Water and bedding were heat-sterilized, whereas food was sterilized by ^{60}Co γ-irradiation. Mice had free access to water and conventional laboratory diet until 24 h before sacrifice. A 12/12 h light/dark cycle was maintained.

Xenografts. LNCaP cells were resuspended in ice-cold Matrigel (1:1) at a final concentration of $3 \bullet 10^6$/0.1 ml. Mice were inoculated in each flank with 0.1 ml of cells using a 26G insulin syringe. The next day, mice were transferred into the gas chamber, where they were treated accordingly.

In vivo measurements of tumor growth. Body weight and tumor volume were measured three times a week during the various treatments. The tumor volume was calculated as length•width•height•0.5236, as measured by a caliper. Data are expressed as the ratio (tumor volume)/(body weight) to compensate different rates of growth in the various experimental situations (**Figure 2**).

Sacrifice. At the end of the observation period, mice were anesthetized by i.p. Na-thiopental (10 mg/100 g body weight) plus heparin (500 units), then they were thoracotomized to

withdraw a blood sample into a heparinized syringe from the left ventricle, and tumors were quickly excised from surrounding skin.

Fig. 2. Picture of a mouse at the end of the observation window. The tumors are clearly visible on both flanks.

Hemoglobin. Hemoglobin (Hb) concentration was measured in blood, by diluting 10 µl of well-stirred blood in 1 ml of Drabkin reagent, followed by incubation for 30 min at room temperature and absorbance reading at λ=540 nm. The concentration was calculated assuming λ=11.05 cm^{-1} mM^{-1}.

Effects of melatonin i.p.. For this set of experiments, mice xenografted with LNCaP cells as described above were treated with melatonin i.p. (30 µg, 1 mg/Kg), given either dissolved in in 100 µL isotonic saline (n=7) or encapsulated in 100 µL SLN (n=7). Mice treated with 100 µL saline acted as control. Whereas melatonin-saline was prepared fresh, melatonin-SLN was obtained from Nanovector S.r.L., Torino, Italy (Gasco *et al.*, 2007). Timing of treatments is given in ***Figure 3***.

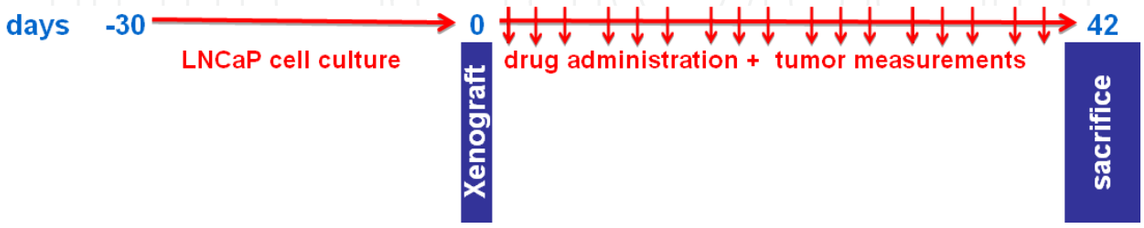
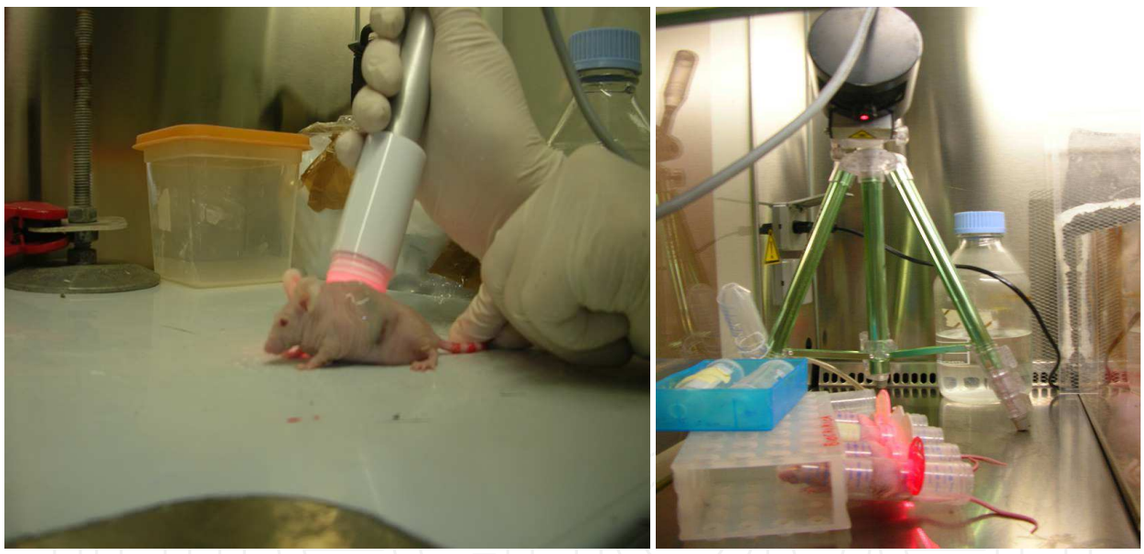


Fig. 3. Timing of melatonin treatments.

Effects of melatonin-laser. For this set of experiments, mice xenografted with LNCaP cells as described above were exposed to laser treatment with (n=11) or without (n=11) melatonin. Melatonin was prepared fresh every week by emulsifying 0.048 mg melatonin/mL of 1.5 % hydroxymethylcellulose for 7 min with a Ultraturrex at the maximum speed in ice and dark.

Then, 15 mL of the suspension or 0.72 mg melatonin was transferred in suitable devices and frozen at -20°C overnight. Each frozen stick (*Figure 4A*) was used to treat 6 mice.
The final dose administered topically was about ~0.120 mg melatonin/mouse/treatment, i.e. 4 mg/Kg. For the administration, the stick containing frozen melatonin (or saline for the control group) was delivered topically for 2.4 min in the correspondence of the xenografts with the frozen stick connected to the laser beam. The duration of the treatment was kept within 2.4 min, because topical application of the frozen stick in small-size animals causes hypothermia (when necessary, mice were kept on a heating plate at 37°C during the treatment, not shown). The concentration of melatonin was selected in order to deliver the wanted amount in 2.4 min. After this first phase, mice were placed in a home-made device, immobilized and exposed for 15 min to the laser scan (the wide of the laser beam scan was set to the minimum value, so to cover only the small tumor area exposed on the mice back) (*Figure 4B*). We used an instrument LASERICE Med C.I.R.C.E. S.r.L., Magnago, Milano (230 V, 50 Hz, 150 mA) constituted by a device for freezing the drug emulsion and by a scanner connected to photodiode laser bean with λ= 635 nm, maximum power <5 mW, collimation lens <20 mV. For our experiments, we selected the software designed for veterinary use in small animals. The software automatically selects the most suitable laser power and frequency to target the drug at the right depth into the tissues.



A B

Fig. 4. (A) Topical administration of melatonin by a frozen stick connected to a low energy laser beam. (B) Experimental setup showing exposure of immobilized mice to high-energy laser scan.

Effects of hypoxia with/out reoxygenation. Data relative to this set of experiments have been published in (Terraneo *et al.*, 2010) and are here reported to enable comparing with the groups with melatonin. Briefly, mice xenografted with LNCaP cells as described above were exposed to either chronic hypoxia (10% O_2) or hypoxia with reoxygenation (3 times/week for 1 h), with normoxia as control (n=17, 19 and 20, respectively). Hypoxia was induced by using the hypoxic chambers described elsewhere that prevent any unwanted contact of the animal with room air during cleaning operations and sacrifice (Milano *et al.*, 2002).

Statistics. Data are expressed as mean±SEM. Significance level was P=0.05 (two-tailed). To detect differences among the groups, we performed one-way ANOVA. If this test resulted significant, the differences between selected pairs of data were tested using the Bonferroni procedure (Instat 3, GraphPad software).

3. Results

Table 3 shows the main characteristics of the mice considered in this study. No significant differences were detected among the various melatonin groups as far as the changes in body weight and Hb concentration are concerned. By contrast, hypoxia depressed the gain in body weight and increased the blood Hb concentration, as expected. The marked decrease in body weight in the mice exposed to hypoxia forced us to shorten the 42-day observation window to 28 days.

	n Surviving/total	*Body weight at entry, g*	*Final body weight, g (42 days of treatment)*	*Blood [Hb], g/L*
Effects of melatonin i.p.				
Saline	6/6	26.63±0.20	26.77±0.80	120±3
Melatonin-saline	7/7	27.71±0.32	32.17±0.46*#	119±6
Melatonin-SLN	7/7	27.96±0.25	31.94±0.90*#	119±3
Effects of melatonin laser				
Laser	11/11	27.24±0.31	29.70±0.91*	126±11
Laser+melatonin	11/11	27.40±0.30	30.58±0.74*	119±6
Effects of hypoxia (28 days of treatment)				
Normoxia	19/20	26.83±0.39	31.17±0.61*	134±3
Chronic hypoxia	16/17	28±0.46	27.38±0.52#	197±3#
Hypoxia with reoxygenation	9/9	27.55±0.37	23.92±0.28*#	205±5#

Table 3. Main characteristics of the mice considered in this study. Data from the hypoxia groups are published in (Terraneo *et al.*, 2010). *, P<0.05 with respect to body weight at entry (unpaired two-tailed Student's t-test); #, P<0.05 with respect to the relative control (ANOVA and Bonferroni post-test).

3.1 Effect of melatonin i.p.

This set of experiments aims at assessing whether the traditional way to administer melatonin results into an oncostatic situation, and whether administration of the same amount of melatonin is oncostatic as well. The xenograft rate of success was in the range 55-90%. Whereas **Figure 5A** reports the time course of the successful xenografts, **Figure 5B** reports the time after the xenograft necessary for the xenografted tumors to be palpable. Data confirm that i.p. treatment with melatonin in saline has important oncostatic potential. Of interest, melatonin does not delay appreciably the time of appearance of the tumors after the xenograft, but rather it decreases the growth rate. Melatonin in SLN has less oncostatic potential than melatonin in saline when given in the same amount, probably as the result of less delivery efficiency than in the melatonin-saline group.

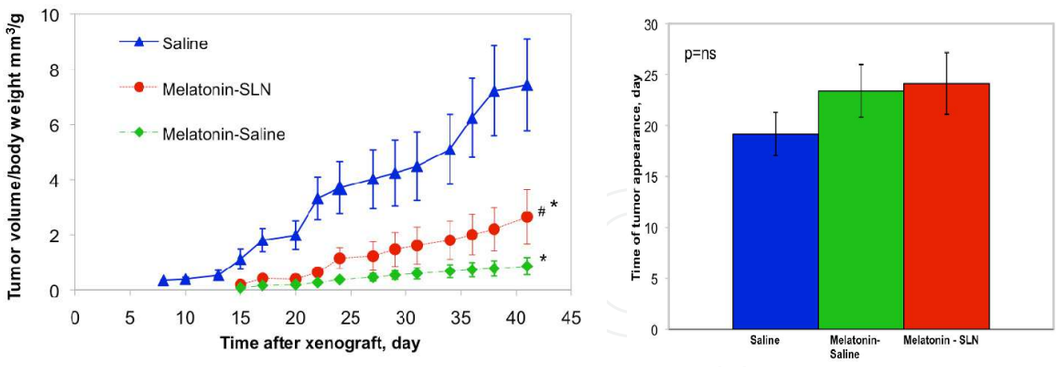


Fig. 5. Time course (A) and time necessary for the tumors to be palpable (B) in mice treated i.p. with either melatonin in saline or melatonin in SLN (0.51 mg melatonin/mouse in 17 administrations over a 42 day period), whereas saline-treated mice represent the control group. Data are expressed as mean ±SEM from 12, 10 and 8 tumors, respectively. *, P<0.05 from untreated; #, P<0.05 from melatonin SLN (ANOVA and Bonferroni post-test).

3.2 Effect of melatonin laser

This set of experiments aims at assessing whether administering melatonin topically by treatment with laser results into an oncostatic situation as that described above for i.p. melatonin. The xenograft rate of success was in the range 75-95%. Whereas **Figure 6A** reports the time course of the successful xenografts, **Figure 6B** reports the time after the xenograft necessary for the xenografted tumors to be palpable. Whereas laser+melatonin results into a oncostatic situation (compare with the saline-treated as reported in Figure 4), it is difficult to discern an effect due to melatonin in comparison with that driven by laser+melatonin.

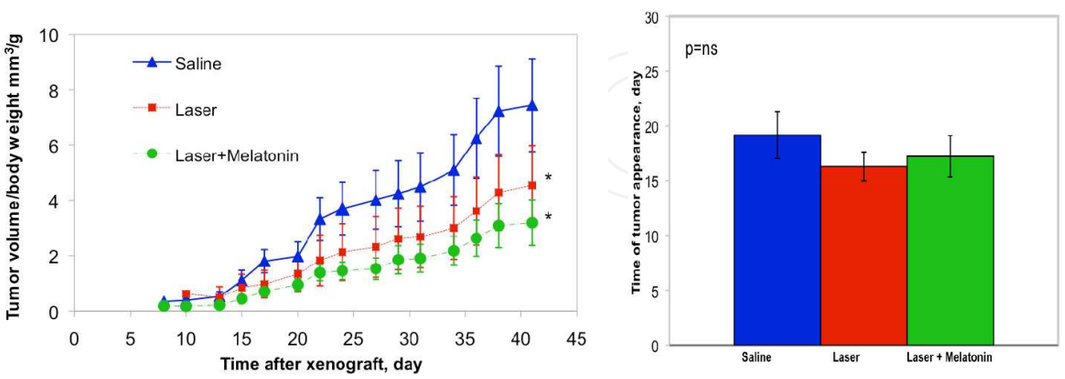


Fig. 6. Time course (A) and time necessary for the xenografted tumors to be palpable (B) in mice exposed to either laser only or laser+melatonin (2.04 mg melatonin/mouse in 17 administrations over a 42-day period). Data are expressed as mean±SEM from 12, 13 and 12 tumors, respectively. No significant difference between the two treatments was observed, but both laser groups are different from Saline control (*, P<0.05).

3.3 Effect of hypoxia

This set of experiments aims at assessing whether inducing local hypoxia increases the growth of prostate tumors as published elsewhere (Terraneo *et al.*, 2010). By decreasing arterial PO_2 from 85 to 34 mmHg, the selected hypoxia severity (10% O_2, equivalent to an altitude of about 5000 m) decreases the total O_2 arterial content by 35%, despite the increased blood Hb concentration. The xenograft success rate was 56-83% without any significant effect of hypoxia nor its way of administration. **Figure 7A** reports the time course of the successful xenografts, and **Figure 7B** reports the time after the xenograft necessary for the tumors to be palpable. Chronic hypoxia resulted into significant increase of the tumor growth rate. Interestingly, an operation aimed at increasing the cytosolic abundance of HIF-1α by approximately 10 times, e.g., exposing mice to repeated reoxygenation events during hypoxia, did not affected appreciably the tumor growth rate. This suggests that mechanisms other than those mediated by HIF-1α might have determined the increased tumor growth rate in hypoxic mice.

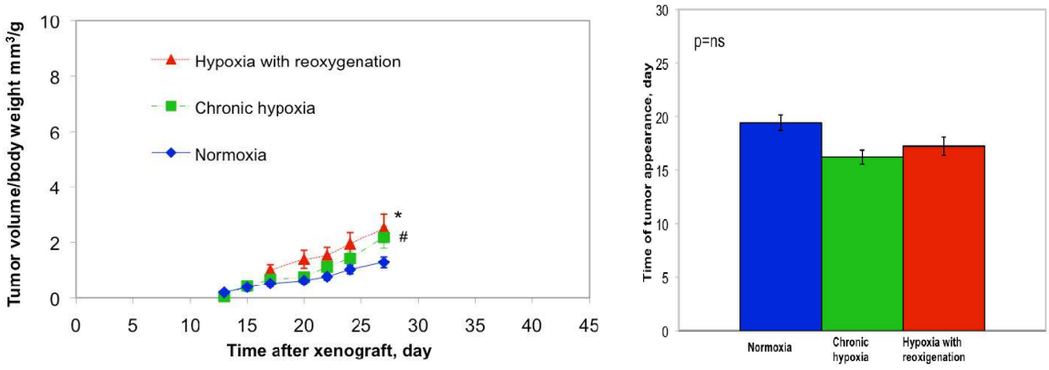


Fig. 7. Time course (A) and time necessary for the xenografted tumors to be palpable (B) in mice exposed to either laser only or laser+melatonin (n g melatonin/mouse in n administrations over a 42 day period). Data are expressed as mean±SEM from n, n and n tumors, respectively. *, P<0.05 from normoxia (ANOVA and Bonferroni post-test).

4. Discussion

Melatonin. After the first observation that in MCF-7 cells *in vitro*, 1 nM melatonin reduces tumor cells invasiveness in Falcon invasion chambers (Cos *et al.*, 1998), the oncostatic properties of melatonin have been thoroughly investigated. Melatonin acts synergistically with castration in inhibiting growth of androgen-sensitive LNCaP tumor through opposite changes in cyclin D1 levels induced by activated MT1 and EGF receptors (Siu *et al.*, 2002). As MT1 receptors are clearly involved in androgen-sensitive LNCaP, but not in androgen-insensitive PC-3 cells (Xi *et al.*, 2001), it is likely that the antiproliferative action of melatonin in LNCaP tumor growth is associated with MT1 receptor protein expression. Furthermore, the clear relationship found in MCF-7 xenografts between melatonin and telomerase activity, responsible of telomere elongation that activated in most human cancers (Leon-Blanco *et al.*, 2003), suggests that melatonin also influences telomerase decreasing its activity in the tumors. Although melatonin at physiologic concentration has no impact on VEGF

expression in three human cancer cell lines (PANC-1, HeLa and A549), at high pharmacological concentrations it markedly reduces expression of VEGF and HIF-1α induced by $CoCl_2$ in cultured cancer cells (Dai *et al.*, 2008). A breakthrough in understanding the mechanisms underlying the oncostatic properties of melatonin came along with unraveling the relationship between melatonin and the hypoxia signaling path. Melatonin indeed inhibits HIF-1α protein expression in normoxic and hypoxic DU145, PC-3 and LNCaP prostate cancer cells without affecting HIF-1α mRNA levels (Park *et al.*, 2009). In HCT116 human colon cancer cells, melatonin destabilizes HIF-1α, suppresses its transcriptional activity, thereby decreasing VEGF expression (Park *et al.*, 2010). In turn, these features block in vitro tube formation and invasion and migration of human umbilical vein endothelial cells induced by hypoxia media, indicating that melatonin plays a pivotal role in tumor suppression via inhibition of HIF-1α-mediated angiogenesis. Our observation that melatonin inhibits LNCaP prostate cancer growth as opposite to enhanced growth in hypoxia agrees with the described findings.

Chronic hypoxia. Despite the acknowledged role of hypoxia in cancer biology, mainly acquired through *in vitro* and clinical studies, accurate analysis of existing literature revealed that the effects of *chronic* hypoxia *in vivo* had not been investigated experimentally as well. To mimic chronic hypoxia, we recently developed an experimental model whereby prostate tumor-bearing mice are exposed to various forms of hypoxia *in vivo*, while continuously monitoring tumor growth and finally assessing the molecular and cellular phenotypes (Terraneo *et al.*, 2010). We found that, although hypoxia *in vivo* promotes prostate cancer growth, this could not be entirely ascribed to HIF-1α, in the favor of other molecular pathways, such as those involving phosphatidyl inositol-3-phosphate/protein kinase B (Akt) pathway. This finding bears important implications especially when designing effective therapies: as a matter of facts, on clinical ground therapies targeting HIF-1α do not appear particularly successful (Fox *et al.*, 2011). Comparing the effects led by chronic hypoxia with/out reoxygenation contributed to univocally assess the latter issue.

Chronic hypoxia with reoxygenation. Reoxygenating the tumors during hypoxia increased HIF-1α cytosolic level >10-fold more than in chronic hypoxia, yet tumor growth was essentially similar to that of chronic hypoxia (Terraneo *et al.*, 2010). Higher HIF-1α in reoxygenated tumors with respect to chronic hypoxia could be a consequence of shorter normalization time since the last exposure to hypoxia (2-3 days), or of reoxygenation-induced HIF-1α stabilization or enhanced synthesis. As the reoxygenation of hypoxic tissues causes considerable oxidative stress (Milano *et al.*, 2004), the associated enhanced generation of mitochondrial ROS may stabilize HIF-1α (Chandel *et al.*, 2000). It has also been proposed that the persistent oxidative stress promoted by ROS during the reoxygenation further amplifies HIF-1α activation in a feed-forward mechanism through a mechanism involving mTOR (Semenza *et al.*, 2007). Therefore, although this finding was key to suppose non-centrality of HIF-1α in cancer growth, the effect of HIF-1α on metastatic potential is still to be investigated.

Therapeutic potential and future perspectives. The role of melatonin as anti-proliferative factor in the management of prostate cancer is currently under study by several research groups. However, its signaling mechanism and its crosstalk with other positive or negative growth regulator factors as HIF-1α, sex steroids, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and others is still under debate and deserves to be thoroughly investigated. Moreover, to elucidate the biological effects of melatonin under

physiological and pathological conditions, its putative antioxidant action is to be studied. Finally, the effect of melatonin on sarcosine (N-methyl derivative of the amino acid glycine), a potentially important metabolic intermediary of cancer cell invasion and aggressivity (Sreekumar *et al*., 2009) merits future attention.

5. Conclusion

In vivo systemic hypoxia promotes prostate cancer growth regardless of HIF-1α expression level and neovascularization, suggesting an important role for hypoxia-dependent pathways that do not involve HIF-1α, as the phosphatidyl inositol-3-phosphate signaling cascade. Melatonin experiments may not only provide a useful probe to assess the effects of HIF-1α on tumor growth, but may also represent the basis for the future introduction of this natural molecule as adjuvant active component in novel therapeutic strategies for the treatment of malignant prostate cancer in humans, for the prevention of cancer relapses, or simply for the amelioration of the quality of life of the oncologic patient.

6. Acknowledgments

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7. References

Allegra, M., Reiter, R.J., Tan, D.X., Gentile, C., Tesoriere, L.& Livrea, M.A. (2003) The chemistry of melatonin's interaction with reactive species. *J Pineal Res* 34(1)**:** 1-10.

Beyer, C.E., Steketee, J.D.& Saphier, D. (1998) Antioxidant properties of melatonin--an emerging mystery. *Biochem Pharmacol* 56(10)**:** 1265-1272.

Brown, J.M.& Giaccia, A.J. (1998) The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res* 58(7)**:** 1408-1416.

Brzezinski, A. (1997) Melatonin in humans. *N Engl J Med* 336(3)**:** 186-195.

Burgess, H.J., Sletten, T., Savic, N., Gilbert, S.S.& Dawson, D. (2001) Effects of bright light and melatonin on sleep propensity, temperature, and cardiac activity at night. *J Appl Physiol* 91(3)**:** 1214-1222.

Cardinali, D.P., Golombek, D.A., Rosenstein, R.E., Cutrera, R.A.& Esquifino, A.I. (1997) Melatonin site and mechanism of action: single or multiple? *J Pineal Res* 23(1)**:** 32-39.

Chandel, N., McClintock, D., Feliciano, C., Wood, T., Melendez, J., Rodriguez, A.& Schumacker, P. (2000) Reative oxygen species generated at mitochondria complex III stabilize HIF-1-alpha during hypoxia: a mechanism of oxygen sensing. *J Biol Chem* 275**:** 25130-25138.

Cho, S.Y., Lee, H.J., Jeong, S.J., Kim, H.S., Chen, C.Y., Lee, E.O.& Kim, S.H. (2011) Sphingosine kinase 1 pathway is involved in melatonin-induced HIF-1alpha inactivation in hypoxic PC-3 prostate cancer cells. *J Pineal Res*.

Cos, S., Fernandez, R., Guezmes, A.& Sanchez-Barcelo, E.J. (1998) Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. *Cancer Res* 58(19)**:** 4383-4390.

Dai, M., Cui, P., Yu, M., Han, J., Li, H.& Xiu, R. (2008) Melatonin modulates the expression of VEGF and HIF-1 alpha induced by CoCl2 in cultured cancer cells. *J Pineal Res* 44(2)**:** 121-126.

Fox, S.B., Generali, D., Berruti, A., Brizzi, M.P., Campo, L., Bonardi, S., Bersiga, A., Allevi, G., Milani, M., Aguggini, S., Mele, T., Dogliotti, L., Bottini, A.& Harris, A.L. (2011) The prolyl hydroxylase enzymes are positively associated with hypoxia-inducible factor-1alpha and vascular endothelial growth factor in human breast cancer and alter in response to primary systemic treatment with epirubicin and tamoxifen. *Breast Cancer Res* 13(1)**:** R16.

Gasco, M.R.& Gasco, P. (2007) Nanovector. *Nanomedicine (Lond)* 2(6)**:** 955-960.

Hockel, M.& Vaupel, P. (2001) Biological consequences of tumor hypoxia. *Semin Oncol* 28**:** 36-41.

Hsing, A.W., Tsao, L.& Devesa, S.S. (2000) International trends and patterns of prostate cancer incidence and mortality. *Int J Cancer* 85(1)**:** 60-67.

Kimbro, K.S.& Simons, J.W. (2006) Hypoxia-inducible factor-1 in human breast and prostate cancer. *Endocr Relat Cancer* 13(3)**:** 739-749.

Korkmaz, A., Reiter, R.J., Topal, T., Manchester, L.C., Oter, S.& Tan, D.X. (2009) Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med* 15(1-2)**:** 43-50.

Kotler, M., Rodriguez, C., Sainz, R.M., Antolin, I.& Menendez-Pelaez, A. (1998) Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J Pineal Res* 24(2)**:** 83-89.

Laudon, M., Gilad, E., Matzkin, H., Braf, Z.& Zisapel, N. (1996) Putative melatonin receptors in benign human prostate tissue. *J Clin Endocrinol Metab* 81(4)**:** 1336-1342.

Leon-Blanco, M.M., Guerrero, J.M., Reiter, R.J., Calvo, J.R.& Pozo, D. (2003) Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both in vivo and in vitro. *J Pineal Res* 35(3)**:** 204-211.

Lewy, A.J.& Sack, R.L. (1996) The role of melatonin and light in the human circadian system. *Prog Brain Res* 111**:** 205-216.

Lupowitz, Z.& Zisapel, N. (1999) Hormonal interactions in human prostate tumor LNCaP cells. *J Steroid Biochem Mol Biol* 68(1-2)**:** 83-88.

Mehnert, W.& Mader, K. (2001) Solid lipid nanoparticles: production, characterization and applications. *Adv Drug Deliv Rev* 47(2-3)**:** 165-196.

Milano, G., Bianciardi, P., Corno, A.F., Raddatz, E., Morel, S., von Segesser, L.K.& Samaja, M. (2004) Myocardial Impairment in Chronic Hypoxia Is Abolished by Short Aeration Episodes: Involvement of K+ATP Channels. *Exp Biol Med (Maywood)* 229(11)**:** 1196-1205.

Milano, G., Corno, A.F., Lippa, S., Von Segesser, L.K.& Samaja, M. (2002) Chronic and intermittent hypoxia induce different degrees of myocardial tolerance to hypoxia-induced dysfunction. *Exp Biol Med (Maywood)* 227(6)**:** 389-397.

Ochoa, J.J., Diaz-Castro, J., Kajarabille, N., Garcia, C., Guisado, I.M., De Teresa, C.& Guisado, R. (2011) Melatonin supplementation ameliorates oxidative stress and inflammatory signaling induced by strenuous exercise in adult human males. *J Pineal Res*.

Pang, S.F., Dubocovich, M.L.& Brown, G.M. (1993) Melatonin receptors in peripheral tissues: a new area of melatonin research. *Biol Signals* 2(4)**:** 177-180.

Park, J.W., Hwang, M.S., Suh, S.I.& Baek, W.K. (2009) Melatonin down-regulates HIF-1 alpha expression through inhibition of protein translation in prostate cancer cells. *J Pineal Res* 46(4)**:** 415-421.

Park, S.Y., Jang, W.J., Yi, E.Y., Jang, J.Y., Jung, Y., Jeong, J.W.& Kim, Y.J. (2010) Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. *J Pineal Res* 48(2)**:** 178-184.

Park, S.Y., Kim, Y.J., Gao, A.C., Mohler, J.L., Onate, S.A., Hidalgo, A.A., Ip, C., Park, E.M., Yoon, S.Y.& Park, Y.M. (2006) Hypoxia increases androgen receptor activity in prostate cancer cells. *Cancer Res* 66(10)**:** 5121-5129.

Reiter, R.J. (1998) Cytoprotective properties of melatonin: presumed association with oxidative damage and aging. *Nutrition* 14(9)**:** 691-696.

Reiter, R.J., Tan, D.X., Manchester, L.C.& Qi, W. (2001) Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochem Biophys* 34(2)**:** 237-256.

Semenza, G.L.& Prabhakar, N.R. (2007) HIF-1-dependent respiratory, cardiovascular, and redox responses to chronic intermittent hypoxia. *Antioxid Redox Signal* 9(9)**:** 1391-1396.

Shiu, S.Y., Li, L., Xu, J.N., Pang, C.S., Wong, J.T.& Pang, S.F. (1999) Melatonin-induced inhibition of proliferation and G1/S cell cycle transition delay of human choriocarcinoma JAr cells: possible involvement of MT2 (MEL1B) receptor. *J Pineal Res* 27(3)**:** 183-192.

Siu, S.W., Lau, K.W., Tam, P.C.& Shiu, S.Y. (2002) Melatonin and prostate cancer cell proliferation: interplay with castration, epidermal growth factor, and androgen sensitivity. *Prostate* 52(2)**:** 106-122.

Sreekumar, A., Poisson, L.M., Rajendiran, T.M., Khan, A.P., Cao, Q., Yu, J., Laxman, B., Mehra, R., Lonigro, R.J., Li, Y., Nyati, M.K., Ahsan, A., Kalyana-Sundaram, S., Han, B., Cao, X., Byun, J., Omenn, G.S., Ghosh, D., Pennathur, S., Alexander, D.C., Berger, A., Shuster, J.R., Wei, J.T., Varambally, S., Beecher, C.& Chinnaiyan, A.M. (2009) Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 457(7231)**:** 910-914.

Tamarkin, L., Baird, C.J.& Almeida, O.F. (1985) Melatonin: a coordinating signal for mammalian reproduction? *Science* 227(4688)**:** 714-720.

Tan, D.X., Manchester, L.C., Reiter, R.J., Qi, W.B., Karbownik, M.& Calvo, J.R. (2000) Significance of melatonin in antioxidative defense system: reactions and products. *Biol Signals Recept* 9(3-4)**:** 137-159.

Terraneo, L., Bianciardi, P., Caretti, A., Ronchi, R.& Samaja, M. (2010) Chronic systemic hypoxia promotes LNCaP prostate cancer growth in vivo. *Prostate* 70(11)**:** 1243-1254.

Vaupel, P. (2004) Tumor microenvironmental physiology and its implications for radiation oncology. *Semin Radiat Oncol* 14(3)**:** 198-206.

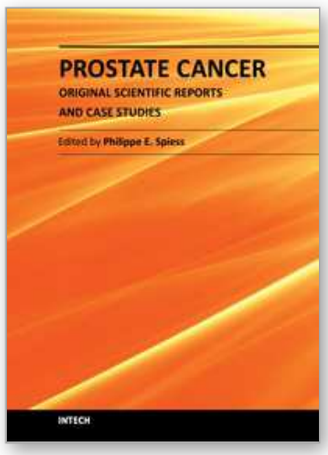
Wilding, G. (1995) Endocrine control of prostate cancer. *Cancer Surv* 23**:** 43-62.

Xi, S.C., Siu, S.W., Fong, S.W.& Shiu, S.Y. (2001) Inhibition of androgen-sensitive LNCaP prostate cancer growth in vivo by melatonin: association of antiproliferative action of the pineal hormone with mt1 receptor protein expression. *Prostate* 46(1)**:** 52-61.

Xi, S.C., Tam, P.C., Brown, G.M., Pang, S.F.& Shiu, S.Y. (2000) Potential involvement of mt1 receptor and attenuated sex steroid-induced calcium influx in the direct anti-proliferative action of melatonin on androgen-responsive LNCaP human prostate cancer cells. *J Pineal Res* 29(3)**:** 172-183.

Zhong, H., De Marzo, A., Laughner, E., Lim, M., Hilson, D.& Zagzag, D. (1999) Overexpression of hypoxia inducible factor 1alpha in common human cancers and their metastases. *Cancer Res* 59**:** 5830-5835.

Zhong, H., Semenza, G.L., Simons, J.W.& De Marzo, A.M. (2004) Up-regulation of hypoxia-inducible factor 1alpha is an early event in prostate carcinogenesis. *Cancer Detect Prev* 28(2)**:** 88-93.



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This book encompasses three sections pertaining to the topics of cancer biology, diagnostic markers, and therapeutic novelties. It represents an essential resource for healthcare professionals and scientist dedicated to the field of prostate cancer research. This book is a celebration of the significant advances made within this field over the past decade, with the hopes that this is the stepping stone for the eradication of this potentially debilitating and/or fatal malignancy.

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