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Intermittent hypoxia and cancer: Undesirable bed partners?

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ABSTRACT

The deleterious effects of intermittent hypoxia (IH) on cancer biology have been primarily evaluated in the context of the aberrant circulation observed in solid tumors which results in recurrent intra-tumoral episodic hypoxia. From those studies, IH has been linked to an accelerated tumor progression, metastasis and resistance to therapies. More recently, the role of IH in cancer has also been studied in the context of obstructive sleep apnea (OSA), since IH is a hallmark characteristic of this condition. Such recent studies are undoubtedly adding more information regarding the role of IH on tumor malignancy. In terms of the IH patterns associated with OSA, this altered oxygenation paradigm has been recently proposed as a determinant factor in fostering cancer incidence and progression from both *in vitro* and *in vivo* experimental models. Here, we summarize all the available evidence to date linking IH effects on several types of cancer.

1. Intermittent hypoxia in cancer

Hypoxia is a frequent occurrence in most solid tumors, and the presence of low intra-tumoral oxygen tensions has been ascribed as a major modifying factor underlying cancer progression, metastasis, angiogenesis, chemoresistance and resistance to irradiation (Muz et al., 2015). In recent years, increasing interest has emerged around intermittent hypoxia (IH) in cancer. Recently, *in vivo* tumor oxygenation assessments in humans have revealed that some tumoral regions can experience cyclic hypoxia (Matsumoto et al., 2010). However, these cycles of hypoxia-re-oxygenation in solid tumors do not manifest predictable regularity and repetitive patterns, and can occur with periodicities from minutes to days. The cyclic hypoxia phenomenon in cancer, and particularly in rapidly growing tumors, has been attributed to changes in solid tumor perfusion, development of newer vascular networks and also, intermittent and aberrant blood circulation (Matsumoto et al., 2010). Taking into account these exploratory evidences, a substantial body of work and investigative effort has been put forth to study the contribution of tumoral hypoxia in the enhancement of some of the malignant properties in several types of cancer (for reviews see refs: DeBerardinis and Chandel, 2016; Dehne et al., 2017; Forster et al., 2017; Manoochehri et al., 2016; Qiu et al., 2017; Tarrado-Castellarnau et al., 2016; Toth and Warfel, 2017; Wong et al., 2016).

In parallel with such discoveries, and based on the conceptual framework that a disease characterized by IH could modify the clinical trajectories of some cancers, efforts have been conducted in recent years to evaluate the role of IH in cancer progression in the context of obstructive sleep apnea (OSA) (Almendros et al., 2012a). In this set of ever expanding studies, the IH patterns applied have been selected to include specific characteristics in order to mimic this sleep disorder (Dewan et al., 2015). In particular, the severity of hypoxia is less severe as far as P_aO_2, but includes a much higher frequency of cycles of hypoxia and re-oxygenation, similar to the ranges observed in actual patients with moderate to severe OSA. In addition, the application of IH in OSA-related cancer studies is delivered both to living mice and directly to the cells. Therefore, *in vivo* IH will not only exert local effects in the tumor cells per se, but also can elicit changes in the host immune response, and pro-inflammatory and angiogenic molecules may be released from different tissues and organs contributing to the oncogenic processes.

Most of studies that have thus far focused on OSA-like IH and cancer have revealed increases in the tumor malignant properties and inferential increases in the resistance to cancer therapies. Although the relationship between OSA and cancer can be also modulated by sleep fragmentation as recently illustrated by Hakim et al. (2014), here, we will exclusively review the findings obtained after IH exposures, and describe the several types of cancer that have been investigated to date under IH challenges.

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Table 1
Studies focused on effects of intermittent hypoxia and melanoma. Abbreviations: OSA: Obstructive sleep apnea; VEGF: Vascular endothelial growth factor; HIF: Hypoxia-inducible factor.

Study	Cell line	Intermittent hypoxia pattern	Field of study	Main effects of intermittent hypoxia
Almendros et al. (2012a)	B16F10 (mouse melanoma)	20 s 6% O_2 40 s 21% O_2 6 h/day–14 days	OSA	- Accelerates tumor growth and more extended necrotic areas.
Almendros et al. (2012b)	B16F10 (mouse melanoma)	20 s 6% O_2 40 s 21% O_2 14 days	OSA	- Augments circulating VEGF and tumor vascularization. - Obesity has not synergistic effects with IH.
Almendros et al. (2013a)	B16F10 (mouse melanoma)	20 s 6% O_2 40 s 21% O_2 28 days	OSA	- Increases lung metastasis and mortality.
Almendros et al. (2013b)	B16F10 (mouse melanoma)	30 min 2% O_2 30 min 21% O_2 4 days	OSA	- Melanoma cells presented increased proliferation in co-culture with macrophages.
Eubank et al. (2013)	B16F10 (mouse melanoma)	80 s 5% O_2 160 s 21% O_2 13 days	OSA	- Increases lung metastases, tumor vascularization and extended necrotic areas.
Li et al. (2016)	B16F10 (mouse melanoma)	Non specified	OSA	- Increases lung metastases which are partially blocked by antioxidant tempol.
Martinive et al. (2006)	B16F10 (mouse melanoma)	1 h 7% O_2 30 min 21% O_2 (x3 cycles)	Cancer	- Reduces radiotherapy-induced apoptosis.
Perini et al. (2016)	B16F10 (mouse melanoma)	30 s 7% O_2 30 s 21% O_2 8 h/day–14 days	OSA	Increases markers of melanoma aggressiveness.
Rofstad et al. (2010)	A-07 (human melanoma)	*In vivo:* 10 min 8% O_2 10 min 21% O_2 (x12 cycles) *In vitro:* 30 min 8% O_2 30 min 21% O_2 (x6 cycles)	Cancer	- Increases blood perfusion, vascularization and lung metastasis. - Upregulates HIF and VEGF expression. - Upregulates of VEGF *in vitro*. - Pre-exposure of A-07 cells to IH *in vitro* did not increased lung colonization potential.

2. Effects of intermittent hypoxia on different types of cancer

2.1. Melanoma

Melanoma is one of the most studied models of cancer when examining the harmful effects of IH on tumor malignancy (Table 1). In the first published study focused on tumor hypoxia, Rofstad et al. investigated whether exposure of melanoma cells to cyclic hypoxia could change its metastatic potential (Rofstad et al., 2010). These investigators employed a humanized model of mice (BALB/c nu/nu) bearing A-07 human melanoma xenografts. The day after tumor cell inoculation, the mice were exposed to 12 cycles of 20 min that alternated 8% O_2 with room air until the tumor xenografts reached a volume of 100 mm^3. Interestingly, the primary tumors of mice exposed to cyclic hypoxia presented higher blood perfusion and vascularization and also resulted in an increased number of lung metastases. These investigators also proposed that the increased vascularization observed in IH-exposed mice could be mediated by the upregulation of vascular endothelial growth factor (VEGF), and primarily mediated by hypoxia-inducible factors (HIFs). The authors concluded that the increased vascularization and blood perfusion that emerged under cyclic hypoxia could facilitate tumor cell intravasation into circulation explaining the increased lung metastasis under these conditions. In addition to the *in vivo* experiments, Rofstad et al. observed that melanoma cells exposed to cyclic hypoxia *in vitro* (6 cycles of 30 min of 8% O_2 alternating with 30 min at 21% O_2) increased VEGF production when compared to continuous normoxia. However, exposure of A-07 melanoma cells to cyclic hypoxia prior to inoculation failed to increase the number of metastases in mice. Furthermore, Martinive et al. showed that murine melanoma cells exposed to IH (three cycles of 1 h 7% O_2 alternating with 30 min 21% O_2) showed reduced magnitude of radiotherapy-induced apoptosis of melanoma cells (Martinive et al., 2006).

In the context of OSA, Almendros et al. published 3 separate studies employing melanoma as an experimental model to study tumor growth (Almendros et al., 2012a), metastasis (Almendros et al., 2013a) and potential interactions with obesity (Almendros et al., 2012b). These studies corroborated that high frequency IH, such as occur in OSA, promotes similar effects on melanoma as previously reported by Rofstad and co-workers (Rofstad et al., 2010). Using a mouse melanoma model (B16F10), Almendros et al. exposed tumor bearing mice to IH (20 s 6% O_2 followed by 40 s 21% O_2), 6 h per day. After 14 days, tumors of mice exposed to IH exhibited a two-fold increase in weight compared to those exposed to intermittent room air (Almendros et al., 2012a). However, despite the original assumptions obesity did not exert synergistic effects with IH, suggesting that OSA and obesity are concurrent enhancers of tumor growth but do not do not promote each other to further tumor malignant properties among obese patients (Almendros et al., 2012b). Interestingly, a clinical study showed that the relationship OSA-cancer was more robust in non-obese patients, suggesting that obesity may mask the harmful effects of OSA on cancer (Nieto et al., 2012). In addition, in lean mice exposed to IH increased tumor vascularization and VEGF levels were uncovered. In a subsequent study, IH for 28 days increased metastatic lung dissemination from the primary inoculum of melanoma tumors (Almendros et al., 2013a). In this model, melanoma cells were more likely to leave the site of the primary tumor, intravasate to the circulation, resist anoikis, evade