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THEORETICAL REVIEW

Obstructive sleep apnea and cancer: Epidemiologic links and theoretical biological constructs

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SUMMARY

Sleep disorders have emerged as highly prevalent conditions in the last 50–75 y. Along with improved understanding of such disorders, the realization that perturbations in sleep architecture and continuity may initiate, exacerbate or modulate the phenotypic expression of multiple diseases including cancer has gained increased attention. Furthermore, the intermittent hypoxia that is attendant to sleep disordered breathing, has recently been implicated in increased incidence and more adverse prognosis of cancer. The unifying conceptual framework linking these associations proposes that increased sympathetic activity and/or alterations in immune function, particularly affecting innate immune cellular populations, underlie the deleterious effects of sleep disorders on tumor biology. In this review, the epidemiological evidence linking disrupted sleep and intermittent hypoxia to oncological outcomes, and the potential biological underpinnings of such associations as illustrated by experimental murine models will be critically appraised. The overarching conclusion appears supportive in the formulation of an hypothetical framework, in which fragmented sleep and intermittent hypoxia may promote changes in multiple signalosomes and transcription factors that can not only initiate malignant transformation, but will also alter the tumor microenvironment, disrupt immunosurveillance, and thus hasten tumor proliferation and increase local and metastatic invasion. Future bench-based experimental studies as well as carefully conducted and controlled clinical epidemiological studies appear justified for further exploration of these hypotheses.

Introduction

The increased prevalence of sleep disorders across the globe has been paralleled by a multitude of studies unraveling potential associations between sleep and diseases affecting nearly every organ system. As part of such trends, the last 15 y have witnessed increased awareness to the possibility that sleep duration and circadian perturbations may be epidemiologically linked to increased risk for developing cancer or, alternatively, promoting adverse cancer outcomes. In addition, similar inferences have more recently emerged in the context of sleep-disordered breathing in general, and more specifically, in relation to the obstructive sleep apnea syndrome (OSAS), whereby two major components of OSAS, namely intermittent hypoxia (IH) and sleep fragmentation (SF) may contribute to altered cancer epidemiology and outcomes.

Here, we will perform an up-to-date critical review of the evidence supporting such possible associations of IH and SF, and further explore any biological mechanisms that may account for such observations. Considering the wide spectrum of sleep disorder conditions and cancer types, we will narrow the scope of this review and focus on a major thematic question and a minor one, the latter (ii below) being viewed as minor, not because of its inferred importance, but rather due to the scarcity of evidence to date:

i) Does OSAS, and more specifically intermittent hypoxia (IH) during sleep modify tumorigenesis or tumor progression?
ii) Does the disrupted sleep/poor quality sleep that results from a large variety of sleep disorders (including OSAS) alter the oncogenic potential or tumor biology?

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Abbreviations

AHI	apnea hypopnea index
CI	confidence intervals
CPAP	continuous positive airway pressure
IFN	interferon
Epi	epinephrine
HIF	hypoxia inducible factor
IH	intermittent hypoxia
IL	interleukin
LPS	lipopolysaccharide
NADPH	nicotinamide adenine dinucleotide phosphate
NE	nor-epinephrine
ODI	oxyhemoglobin desaturation index
OSAS	obstructive sleep apnea syndrome
ROS	reactive oxygen species
SF	sleep fragmentation
TAM	tumor associated macrophage
TGF	transforming growth factor
TLR	toll-like receptor
VEGF	vascular endothelial growth factor

However, we will not examine the effect of cancer and cancer treatments on sleep quality and duration [1]. Similarly, we will not address in detail the issue of circadian disruption as seen in shift workers and other professions and cancer risk. As discussed in detail in a recent review by Haus and Smolensky [2] and other literature, shift work and other exposures that disrupt the circadian rhythm continuum are associated with epigenetic modifications of several key circadian genes, the latter exerting transcriptional regulatory functions affecting the expression of cancer-related susceptibility genes, as well as playing key roles in gene networks regulating cell division and DNA repair. Therefore, it is not surprising that increased risk for the occurrence of a variety of malignant tumors has been consistently reported among long-term night workers and shift workers [3–7], and that even use of increased nocturnal light exposure potentially reducing melatonin secretion may be accountable for such effects [8], as nicely illustrated by a very recent paper in a murine cancer model [9,10]. Finally, we will not address the potential implications of sleep duration in the epidemiology of cancer. Readers are referred to previously published reviews or meta-analyses on this specific topic [2,11–16]. Although the possibility exists that some of the mechanistic pathways potentially underlying circadian rhythms, sleep disorders, and cancer may be shared with the current focused review (see Fig. 1), we will specifically tackle the biological plausibility and potential theoretical mechanisms underlying the epidemiological associations between disrupted sleep and intermittent hypoxia that characterize OSAS and cancer incidence and cancer outcomes, with the aim to stimulate the field in further exploring this emerging area of investigation.

Epidemiology of OSAS and cancer

Contrary to research in animal models or in cell cultures, where it is relatively easy to control the experimental conditions to avoid effects from confounding variables, investigating the role of nocturnal OSAS in patients is difficult, owing to the co-existence of factors playing already well-known roles in enhancing cancer, such as age, obesity, and sleep duration. Studying what is the potential role of OSAS and its constitutive elements, namely IH, and SF, in boosting the genesis, growth and metastasis of tumors –and their clinical consequences in terms of cancer incidence and mortality- requires large cohorts that are monitored for long periods of time. Very well designed studies involving a reduced number of well-studied patients may also be useful, but they provide just preliminary evidence that must require final confirmation in comprehensive population studies.

Although several patient studies have recently investigated whether sleep breathing disturbances increase the risk of cancer ([17–26]; Table 1), few of these studies have addressed the specific role played by IH, and two of them have been carried with remarkable limitations concerning sleep assessments [18,23,24]. Indeed, Christensen et al. studied 8783 subjects that answered questions about snoring and breathing cessations, and information about daytime sleepiness based on the Epworth sleepiness scale was collected in a subset of the participants. This study showed no relationship between self-reported sleep abnormalities and cancer [18]. However, as discussed by Peppard and Nieto [25], the negative results could be in part caused by inaccuracy in assessing sleep disordered breathing by symptom-based self-reports. The second study with considerable methodological limitations was carried out from a database in Taiwan, including 23,055 patients with OSAS [27]. However, in this study most cases of OSAS were diagnosed only on the basis of clinical symptoms (i.e., persistent snoring and witnessed apnea) and the apnea severity data were not available in the database. However, these authors reported that the overall risk for developing primary central nervous system cancer was significantly higher in the OSAS group, with and adjusted hazard ratio of 1.54 [25]. In an additional study from Canada [26], a diagnosis of OSAS was not associated with increased risk of prevalent or incident cancer. Indeed, over a follow-up period of 7.8 y, 627 (6.5%) of the 9629 OSAS patients who were free of cancer at baseline had incident cancer. Using multivariable regression models, the severity of OSAS was not significantly associated with either prevalent or incident cancer after adjusting for age, sex, body mass index and smoking status at baseline [25]. In contrast, another recent study in women suggested that the risk of breast cancer was increased in those with OSAS [27].

To date, the most solid evidence on the association between IH during sleep and cancer has been obtained from three human population cohorts: the general population-based Wisconsin cohort [17], a multicenter Spanish database of patients with suspected sleep breathing disorders [18,19], and a cohort of Australian patients [22]. The first two populations involved large numbers of subjects and long-term follow-up but suffered from the limitation that, being retrospectively designed (i.e., analyzing already existing cohort data), nocturnal hypoxemia could only be assessed by an index accounting for hypoxemic time instead of an index directly quantifying the number and magnitude of oxygen desaturation events. However, the putative validity of measuring IH using a polysomnographically-derived index has been demonstrated in a recent pilot study that prospectively showed that the rate of melanoma progression is associated with the magnitude of intermittent hypoxemia [23]. The Australian population study, although involving a lower number of patients than the Wisconsin and Spanish cohorts, evaluated OSAS using a desaturation index [22].

The importance of the results obtained from the Wisconsin sleep cohort comes from the fact that it studies patients from the general population. Specifically, the cohort was initiated in 1989 by recruiting subjects, not from patient groups, but rather from different professional jobs in employees of the Wisconsin state government. Another important characteristic of this cohort is that sleep disturbances were diagnosed by full polysomnography. The cancer mortality in this cohort could be derived from more than 1,500 subjects followed-up for a total period of up 22 y, with a median of 18 y. Among a total of 112 deaths in the cohort, 50 were

caused by different types of cancer. However, the analysis of the effect of nocturnal hypoxemia was carried out in the subset of patients for whom the hypoxemia data were available: 1,306 subjects, with 33 deaths from cancer [17]. Since no index directly measuring nocturnal intermittent hypoxemia was available for the whole cohort, to focus on the role played by hypoxia, the authors considered an index of hypoxemia defined as the percent sleep time spent under 90% oxygen saturation. Values for hypoxemia index of 0.8%, 3.6%, and 11.2% were used to defined mild, moderate and severe OSAS, respectively (in the absence of a standard definition, these cutoffs were chosen based on the same quantile distribution such as that derived AHI categories widely used in clinical practice—5, 15, and 30 apneas/hypopneas per hour). The hazard ratios showed a highly statistically significant dose-response relationship (p for trend = 0.0008): patients with a severe sleep breathing disorder (defined by a hypoxemia index ≥11.2%) were 8.6 times more likely to die of cancer than those without OSAS (hypoxemia index<0.8%) even after accounting for possible confounding variables (age, gender, BMI, smoking, diabetes). For comparison, when the AHI was used instead of the hypoxemia index to characterize severe OSAS severity, the cancer mortality comparing those with AHI>30 to those without OSAS was about 4.8-fold higher. Also, when used as a continuous variable, an increase in one log-unit in the hypoxemia index was associated with an adjusted hazard ratio of cancer mortality of 1.9 (95% CI, 1.3–2.9; P = 0.002) [17].

Both cancer incidence and mortality in patients with suspected sleep breathing disorders were studied in two subsequent reports from a multicenter database of adult Spanish patients referred to sleep laboratories because of suspicion of sleep apnea [19,20]. The study involved a sample of more than 5,300 patients followed for a median of 4.5 y. OSAS status was determined by respiratory polygraphy in about two-thirds of the patients; the rest underwent a full overnight polysomnogram. When using the same hypoxemia index as in the Wisconsin study, cancer incidence augmented with increasing levels of OSAS [19]. The adjusted hazard ratio of cancer incidence associated with a 10-unit increase in the hypoxemic index was 1.1 (95% CI, 1.0–1.1). Interestingly, in patients who were untreated over the follow-up the association between the hypoxemic index and cancer incidence was slightly stronger [19]. This patient database was also interrogated for the relationship between nocturnal hypoxemia and cancer mortality [20]. Cumulative cancer mortality increased across hypoxemic index categories. Compared to the lower severity category, the fully adjusted hazard ratio (95% CI) of cancer mortality for the more severe category (hypoxemic index>13%) was 2.06 (1.72–4.58). A significant fully adjusted increase in mortality risk was also observed with increasing log-transformed hypoxemic index as a quantitative variable (1.21 [1.03–1.42]). When only the subgroup of 527 patients with a diagnosis of cancer was analyzed, the hypoxemic index was significantly associated with cancer mortality after adjusting for confounders (HR 1.19, 95%CI 1.02–1.41) [20].

Consistent with the evidence from laboratory and animal experiments discussed in the following sections, the reports on the Wisconsin [17] and Spanish cohorts [19,20] suggest that there is an association between nocturnal hypoxemia and cancer mortality in patients with OSAS. The most important limitation of these studies is that, because they were not specifically designed to determine the unique role of IH in carcinogenesis, and the hypoxemic index that was used as a surrogate of IH did not directly quantify individual hypoxic events, but only assessed the percentage of night time under 90%O_2 saturation in arterial blood. With these data, we cannot exclude that patients with mild or moderate chronic hypoxia (caused by obesity or other diseases) might have been classified as OSAS patients, even though the studies from the Spanish groups excluded patients with respiratory failure or long-term oxygen therapy. Furthermore, results from a recent prospective pilot study have documented a direct association between the aggressiveness of human cutaneous malignant melanoma and an index directly quantifying intermittent hypoxemia [24]. Fifty-six patients with documented data of melanoma aggressiveness (tumor mitotic rate, Breslow index, presence of ulceration, stage of disease and growth rate of melanoma) were subjected to a sleep study and multivariate analyses examined the independent relationship between nocturnal oxygen desaturation indices at 3% (ODI3%) and at 4% (ODI4%)) and the measures of tumor aggressiveness. In fully adjusted multivariate analyses, ODI3% (odds ratio [OR] 1.08, 95%CI 1.02–1.11) and ODI4% (OR 1.1, 95%CI 1.02–1.2) were independently associated with an increased melanoma growth rate. Furthermore, the desaturation indices ODI4% and ODI3% were significantly correlated with other aggressiveness factors of cutaneous malignant melanoma, such as Breslow index, presence of ulceration and mitotic index [24]. This study has the obvious limitations of including a reduced number of patients and the focusing on a single type of cancer that is not a highly prevalent type of tumor. However, the dose-response relationship linking IH and tumor aggressiveness adds further evidence on that derived in previous studies using a less suitable hypoxemic index in large populations and follow-up periods [17,19,20].

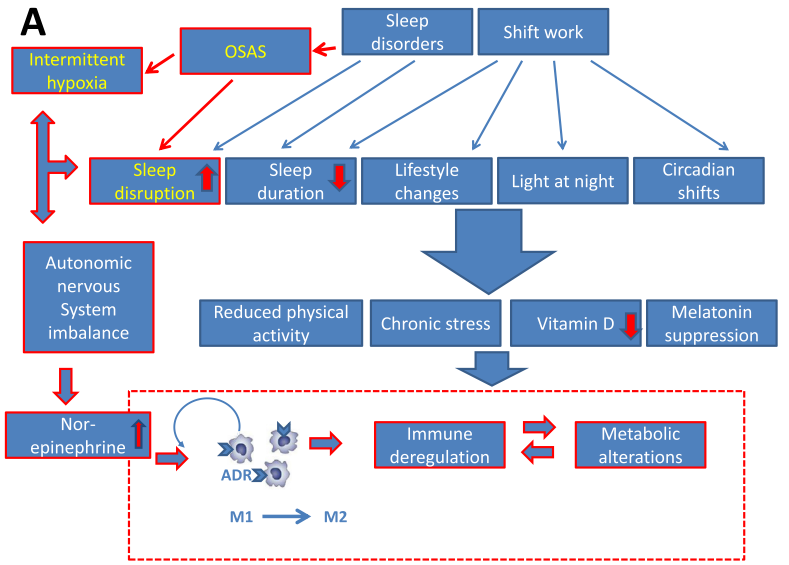
The recent study by Marshall et al. [22] provides additional support to the notion that there is an association between OSAS and cancer risk. Indeed, in this study that summarizes the monitoring of 397 patients for 20 y, the authors reported that using fully adjusted models, moderate-severe OSAS was significantly associated with both cancer mortality and incident cancer, with hazard ratios of 3.4 and 2.5, respectively [22]. Whereas there are data clearly suggesting that in patients there is an association between sleep apnea and cancer risk, studies focused on the potential mechanisms causing this association in humans are so far lacking. However, a recent study has provided some clues in this regard [21]. Indeed, Gharib et al. [21] studied the whole-genome expression of peripheral blood leucocytes from 18 patients with OSAS (AHI>30 event/h) before and after one month of CPAP treatment. Gene set enrichment analysis comparing leucocytes in each patient from pre- and post-CPAP treatment indicated that some gene sets involved in neoplastic processes were down regulated by OSAS treatment, suggesting potentially novel mechanisms linking OSAS and tumorigenesis. Although these can only be considered preliminary findings from a pilot study that would need to be replicated in larger studies, it is noteworthy that the authors identified candidate genes that may be important mediators in the relationship between OSAS and neoplastic diseases [21].

Epidemiological evidence linking sleep quality and cancer

Few epidemiological studies are available and have addressed this specific facet linking sleep quality and sleep duration with risk of cancer. The methods used in these studies to characterize sleep disturbances varied widely as did the cancer outcomes and the results.

The most abundant evidence is in relation to breast cancer. For example, recent meta-analyses consistently show that shift-work is associated with a higher risk of breast cancer [28–30]. These studies should be interpreted with caution, however, as shift-work is not only associated with disturbances of sleep patterns but also with other socio-demographic factors and comorbidities (including obesity and metabolic abnormalities) [31–34] that may act as confounders of this association.

Results from studies on sleep duration in relation to breast cancer risk show inconsistent results. While some studies suggest that short sleep duration is associated with higher risk of breast cancer [35–37] other studies failed to show such an effect [38–41].


A
Intermittent hypoxia
OSAS
Sleep disorders
Shift work
Sleep disruption
Sleep duration
Lifestyle changes
Light at night
Circadian shifts
Autonomic nervous System imbalance
Reduced physical activity
Chronic stress
Vitamin D
Melatonin suppression
Nor-epinephrine
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Immune deregulation
Metabolic alterations
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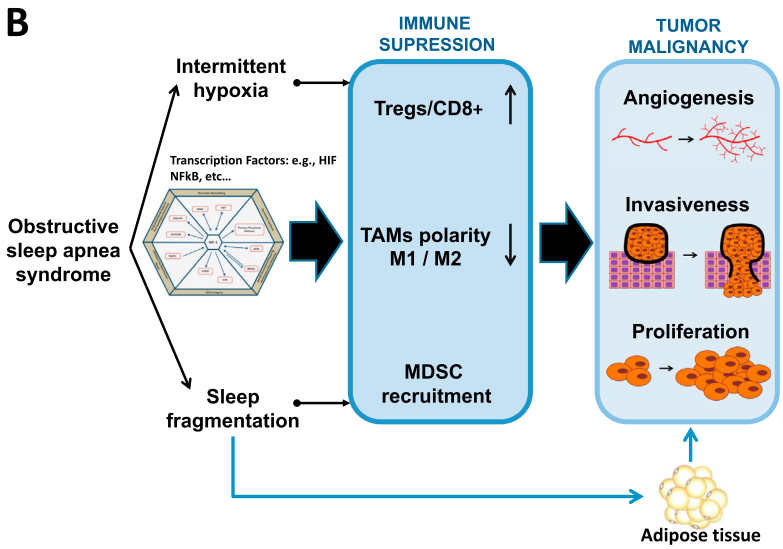
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IMMUNE SUPRESSION
TUMOR MALIGNANCY
Intermittent hypoxia
Transcription Factors: e.g., HIF NFkB, etc...
Obstructive sleep apnea syndrome
Sleep fragmentation
Tregs/CD8+
TAMs polarity M1 / M2
MDSC recruitment
Angiogenesis
Invasiveness
Proliferation
Adipose tissue

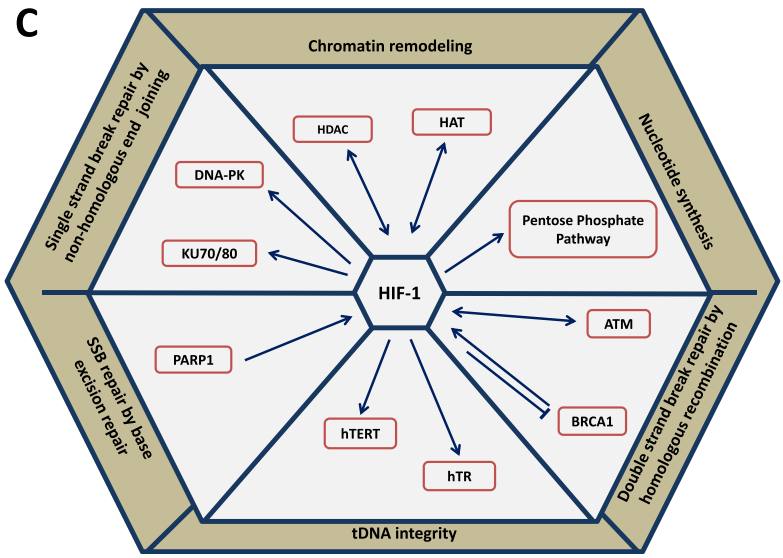
C
Chromatin remodeling
Single strand break repair by non-homologous end joining
Nucleotide synthesis
HDAC
HAT
DNA-PK
KU70/80
Pentose Phosphate Pathway
HIF-1
ATM
PARP1
BRCA1
hTERT
hTR
SSB repair by base excision repair
Double strand break repair by homologous recombination
tDNA integrity

Table 1
Summary characteristics of published studies examining potentials associations between OSA and cancer.

Authors, year [reference#]	Country	n	Sample characteristics	Major findings
Incidence Studies				
Christensen et al., 2013 [18]	Denmark	8,783	Subjects without previous cancer diagnosis surveyed with snoring questionnaire and Epworth scale followed over the period of 1992–2009	No overall association between symptoms of SDB and incident cancer However, in group with high daytime sleepiness, higher cancer incidence (HR: 4.09; 95% CI 1.58–10.55) emerged in persons younger than 50 y
Campos-Rodriguez et al., 2013 [19]	Spain	4,910	OSA patients (confirmed by polygraphy) followed over median follow-up of 4.5 y without initial diagnosis of cancer	TSat(90) was associated with cancer incidence in patients younger than 65 years (adjusted HR, 1.13 [95% CI, 1.06–1.21] per 10-unit increase in TSat(90)) and males (adjusted HR, 1.11 [95% CI, 1.04–1.17] per 10-unit increase in TSat(90)). AHI was not associated with cancer incidence in the adjusted analyses, except for patients younger than 65 y (adjusted HR for AHI >43 vs. <18.7, 1.66; 95% CI, 1.04–2.64).
Kendzerska et al., 2014 [26]	Canada	9,629	OSA patients (confirmed by polysomnography) followed over median follow-up of 7.8 y without initial diagnosis of cancer	In multivariable regression models, after adjustment for age, sex, body mass index and smoking status at baseline, neither AHI (adjusted HR for incident cancer: 1.02, 95% CI 0.80–1.31), nor TSat(90) (adjusted HR: 1.00, 95% CI 0.99–1.02) were associated with increased cancer risk.
Chen et al., 2014 [23]	Taiwan	23,055	OSA patients (confirmed by polysomnography) followed over median follow-up of 10 y without initial diagnosis of cancer	The overall risk for developing primary CNS cancers was significantly higher in the OSA group (adjusted HR: 1.54; $P = 0.046$) after adjusting for age, gender, and obesity, among other variables, and was particularly higher in the insomnia with OSA subgroup (adjusted HR: 2.20; $P = 0.001$) and in the OSA without surgical treatment subgroup (adjusted HR: 1.831; $P = 0.003$).
Chang et al., 2014 [27]	Taiwan	846 OSA women and 4,230 women without OSA	Female OSA patients (confirmed by polysomnography) followed over median follow-up of 5 y without initial diagnosis of cancer	Adjusted HR of breast cancer in patients with OSA was higher [2.09; 95% CI: 1.06–4.12; $P < 0.05$] than in controls
Mortality Studies				
Nieto et al., 2012 [17]	USA	1,522	Community-based sample assessed by polysomnography and followed for up to 22 years	Adjusting for age, sex, body mass index, and smoking, OSA was associated with cancer mortality (HR: 1.1 (CI: 0.5–2.7) for mild OSA (AHI, 5–14.9), 2.0 (CI: 0.7–5.5) for moderate OSA (AHI, 15–29.9), and 4.8 (CI: 1.7–13.2) for severe OSA (AHI $\geq$ 30) (P-trend = 0.0052). For categories of increasing severity of the hypoxemia index, the corresponding relative hazards were 1.6 (CI: 0.6–4.4), 2.9 (CI: 0.9–9.8), and 8.6 (CI: 2.6–28.7) (P-trend = 0.0008).
Martínez-García et al., 2014 [20]	Spain	5,427	OSA patients (confirmed by polygraphy) followed over median follow-up of 4.5 y	Log-transformed TSat(90) was independently associated with increased cancer mortality (HR: 1.21; 95% confidence interval [CI], 1.03–1.42), particularly in patients <65years (HR: 1.73; 95% CI, 1.23–2.4; upper vs. lower TSat(90) tertile: HR: 14.4; 95% CI, 1.85–111.6).

CI : 95% confidence intervals; HR : hazards ratio; TSat(90) : Time spent with SaO2 <90% during sleep.
Abbreviations: AHI - apnea hypopnea index; CNS - central nervous system; CI - confidence intervals; HR - hazards ratio; OSA - obstructive sleep apnea; SDB - sleep disordered breathing; TSat(90) - time spent with SaO2 >90% during sleep.

A recent meta-analysis also did not support the hypothesis that short sleep duration is related to breast cancer risk [16]. One important limitation of these studies is the use of self-reported information on sleep duration, which could lead to misclassification and bias towards the null. In a recent large prospective study among post-menopausal women in Denmark, exposure to road traffic and railway noise (presumably associated with poor quality sleep) found no overall association with breast cancer, but identified a modest statistically significant elevated risk of estrogen receptor negative breast cancer [42].

The evidence with respect to other cancer sites is even sparser at this time. In a large prospective study of more than 140,000 post-menopausal women participants in the Women's Health Initiative that were followed for an average of 11 y, insomnia (measured by a validated 5-item questionnaire) was significantly associated with the incidence of thyroid cancer [43]. However, this association was

Fig. 1. (A) Theoretical framework of possible mechanisms by which sleep duration, sleep continuity, and OSAS might contribute to the epidemiological characteristics of cancer – current review is particularly focused around the areas framed in red color; (B) potential mechanisms affected by either sleep disruption or intermittent hypoxia in the context of OSAS: altered signal transduction and transcription involving multiple ubiquitous transcription factors such as nuclear factor kappa B and hypoxia-inducible factor (HIF) (C) will lead to maladaptive transcriptional regulation potentially affecting not only malignant transformation potential, but also disrupt multiple immunoregulatory cellular targets that in turn will impose changes in oncogenic properties of the tumor (modified from [156–158]). The major concepts shown here indicate that sleep disruption and intermittent hypoxia during sleep can increase sympathetic nervous system outflow, which can not only alter innate cell function by activation of adrenergic receptors, but may further potentiate the role of vasculogenesis and stromal cell support of tumors. Similarly, disrupted sleep and intermittent hypoxia may either directly or via metabolic deregulation modify the phenotype and function of immunologically competent cells such as macrophages (e.g., anti-tumoral M1 macrophages adopting a M2 phenotype that is tumor-supportive). The mechanisms underlying these changes remain for the most part unknown; however, it is very likely that IH and SF alter the regulation of transcriptions factors such as HIFs, nuclear-factor kappa B, etc … all of which may then modify the properties and phenotype of tumor resident cells to alter their functional properties thereby promoting proliferation, invasion, and metastatic potential. Abbreviations: MDSC - mesenchymal derived stem cells; OSAS - obstructive sleep apnea syndrome; TAMs - tumor associated macrophages; Tregs - T regulatory lymhocytes.

limited to non-obese women; furthermore, no association was found between sleep duration and risk of thyroid cancer in this study.

While the associations between short sleep duration and cancer incidence and outcomes have been more extensively explored, the potential effects of perturbed sleep, as illustrated by poor-quality sleep, sleep fragmentation, or sleep discontinuity have been much less extensively investigated, and have recently emerged as being particularly important in the context of tumorigenesis.

Animal models of OSAS

As discussed above, the preponderant focus of the work on sleep and cancer up to the present time has revolved around the impact of cancer therapies on altered sleep quality and attendant quality of life measures [2,44]. Moreover, the degree of sleep disruption that might be present due to underlying sleep disorders, e.g., OSAS, or be environmentally induced by sleep disruptors such as traffic noise has not been specifically addressed. To explore the potential implications of SF on tumor proliferation and invasiveness, we exposed mice to a now extensively validated paradigm of disrupted sleep (SF) whereby mice undergo periodic arousals during their sleep period [45–48]. Although SF exposures do not induce increases of circulating levels of corticosterone suggesting that they are relatively non-stressful, we have not specifically examined to date whether SF alters systemic and tissue levels of catecholamines. Notably, the current SF procedure preserves overall sleep duration, such that disrupted sleep in this murine model occurs in the absence of sleep curtailment. In our experiments, we employed two well-established syngeneic solid tumor models in mice, specifically the TC-1 and 3-LLC cell lines, and assessed in vivo proliferative rates, tumor size, and tumor invasiveness in SF-exposed and normal sleep control mice [49]. The selection of these tumor types was predicated on the established validity of the cancer model in mice rather than follow any specific rationale based on epidemiology, particularly considering that the current data available do not necessarily point to a specific tumor type as being particularly susceptible to sleep disorders. TC1-cell tumor weight was significantly higher and nearly double the size of control mice at day 28 in SF-exposed mice ($p < 0.001$). Similarly, in mice exposed to SF and injected with 3LLC cells, markedly accelerated tumor growth and tumor weight emerged. Histological assessments by blinded investigators revealed that tumors originating form SF-exposed mice were significantly more likely to display invasion of the capsule to neighboring tissues and surrounding tissue destruction. Thus, disrupted sleep such as occurs in OSAS markedly accelerates tumor progression and invasion even in the absence of intermittent hypoxia (IH; see below). More recent work from our laboratory has further indicated that in sharp contrast with other tissues, such as brain and adipose tissues [46,50–52], in which SF promotes increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, the opposite, i.e., reduced NADPH oxidase activity and expression occur within the solid tumor microenvironment, and underlie components of the increased aggressiveness of the tumor under SF conditions [53]. These recent findings have prompted substantial interest and should lead to enhanced research efforts [54].

1) Cancer and the sympathetic-catecholaminergic system:

1a) Sleep, sleep fragmentation, and sympathetic outflow:

Sleep and sleep stage have now been firmly established as regulating sympathetic outflow. In the transition from wakefulness to sleep, there is a shift toward a more predominant parasympathetic balance, which increases as the depth of NREM sleep increases [55]. During REM sleep, tonic sympathetic outflow is increased and resembles the levels found during wakefulness [56]. Even though plasma levels of the sympathetic neurotransmitters show a similar pattern [57], it is unclear whether the actual levels of catecholamines released from the tissue-located nerve terminals oscillates in a parallel fashion. This is an important lacuna in our level of knowledge that will have obvious implications as discussed below. During arousals from sleep, surges in sympathetic outflow occur and can lead to major changes in systemic and local tissue catecholamine levels. Furthermore, the critical importance of the adrenergic system to arousal and sleep state regulation has been studied in depth [58–60]. However, although the overall impact of SF on sympathetic activity and catecholamine synthesis and release has not been thoroughly investigated, initial evidence supporting increases in sympathetic outflow have been reported in humans subjected to SF for relatively short periods of time [61–65].

1b) IH and sympathetic outflow:

Similar to the aforementioned considerations elaborated in the context of alterations in sympathetic outflow elicited by perturbations in sleep, the potential contributions of such alterations in sympathetic activity in the context of IH cannot be overlooked. Indeed, a substantial body of evidence has accumulated over the last 25 y to indicate that IH is a powerful recruiter of sympathetic outflow both centrally and peripherally, and that sustained hypoxia and intermittent hypoxia elicit substantially different patterns of catecholaminergic synthesis and release [66–73]. However, the effects of IH on intra-tumoral levels of norepinephrine (NE) or epinephrine (Epi), or the potential changes in macrophage-driven production of catecholamines and the consequences of such changes as far as macrophage expression of adrenergic receptors and of macrophage polarity have not yet been explored. As indicated above, macrophages can synthetize and release catecholamines, which not only can influence in a very selective way the biological activity and properties of surrounding neighboring cells, but can further modulate their own activity, polarity, and fate in a contextually-appropriate mode through ligand mediated activation of macrophage surface adrenergic receptors [74–77].

1c) Sympathetic outflow and cancer:

The biologic effects of NE and Epi are mediated by the coordinated actions elicited by the ligation of these compounds to α1-, α2-, and β-adrenergic receptor families. These receptors are distributed according to distinct patterns in almost every organ and tissue in the body, and selectively activate multiple biochemical pathways [78]. It is of particular relevance that all β-adrenergic receptor subtypes are expressed in many sites of tumor growth and metastasis, and that their downstream signaling pathways regulate the function of several cancer-relevant cellular substrates such as epithelial cells, vascular myocytes and pericytes, adipocytes, fibroblasts, neural and glial cells, and most of the lymphoid and myeloid immune cells [79,80]. These observations have prompted the examination of a potential causative link between stress, particularly chronic stress, and cancer outcomes. Inferential data over the last decade appear to be overall supportive of such connection [81], whereby several studies have indicated that long-term treatment with β-blockers reduces the prevalence and particularly ameliorates the outcome of several cancers in humans [82–89]. In vitro studies have shown that adrenergic signaling, particularly β-adrenergic receptor signaling, can regulate multiple cellular processes involved in cancer

progression, including tumor cell proliferation, extracellular matrix invasion, angiogenesis, activation of matrix metalloproteases, and notably expression of inflammatory and chemotactic cytokines [90–92]. Among the latter, increased recruitment of macrophages into the tumor [93], with increased expression of pro-inflammatory cytokines such as IL-6 and IL-8 by tumor cells [91,94] lead to marked enhancements in tumor invasion and metastasis. Recent work has also implicated adrenergic receptors in the expression and functional binding and activity of critical innate immunity toll-like receptors such as TLR2 and TLR4 [95]. Furthermore, macrophages and monocytes can synthetize and release catecholamines that will further modulate surrounding cellular substrates or bind and activate specific adrenergic receptors on the macrophage surface to induce selectively regulated changes in their functional status [96–101]. Thus, emergent evidence points to a major role played by sympathetic outflow on changes in macrophage recruitment and differentiation that alter gene expression within the primary tumors [93], and assuming that such changes do indeed occur in sleep disorders, they provide a fertile ground for future research on the intricate connectivity between sleep, adrenergic signaling, and cancer biology. We should also point out that epidemiological studies appear to corroborate the putative role of adrenergic receptors in the context of not only tumor biology but also outcomes. Indeed, epidemiologic studies have shown that use of β-blockers, inhibiting β-adrenergic receptor activity, is associated with reduced prostate cancer-specific mortality, and some of the mechanisms underlying the anti-metastatic effects of adrenergic receptor blockers are now being uncovered [102–107].

2) Cancer and immunity:

It is now well established that the immune system in general, and macrophages in particular, participate in multiple cancer-related processes. Among macrophages, 3 major sub-type classifications have been thus far enunciated, with classically activated macrophages such as after stimulation with lipopolysaccharide (LPS or interferon (IFN)-γ being designated as M1, alternatively-activated wound-healing macrophages resulting from stimulation with IL-4 or IL-13 being termed M2a, and a macrophage phenotype derived from IL-10 or transforming growth factor (TGF)-β stimulation being termed regulatory macrophage or M2b. Alteration in the polarity of macrophages has now been clearly established as a major determinant of tumor properties. The tumor microenvironment delivers various signals that shape macrophage phenotypes into preferentially promoting tumor growth instead of attacking tumor cells [108]. Simplistically stated, pro-inflammatory cytokines released by M1 macrophages exert anti-tumoral functions, while tissue regenerative properties of M2 macrophages (e.g., angiogenesis, stromal signaling) support tumor proliferation and invasion. New therapy approaches are tested that re-educate tumor-associated macrophages or block the tumor-promoting function in these cells, demonstrating further the potential of macrophage polarization in this setting. Tumor-associated macrophages (TAMs) have now been identified as critically important constituents of cancer micro-environment, where they contribute to tumor-related processes through the release of growth factors, cytokines, inflammatory mediators, and proteolytic enzymes implicated in tumor growth and invasion.

In recent years, interest in the role played by tumor-associated neutrophils has also arisen. Indeed, production of atypical T cell-suppressive neutrophils occurs during early tumor progression, at the onset of malignant conversion, and innate immune cells appear to preferentially accumulate in peripheral sites of tumors where they may underlie aspects of tumor migration, invasion and metastasis [81,109–114].

2a) SF, immune deregulation and cancer:

As mentioned above, application of SF during sleep promoted accelerated TC1 and 3LL tumor growth [49,54]. Thus, we further sought to discern whether SF directly promotes tumor cell proliferation, or whether TAMs play a role in the increased tumor size and invasion observed in mice exposed to SF during sleep [49]. We found that TAMs expressing M2 macrophage markers were increased in number in TC-1 tumors derived from mice exposed to SF, and that the majority of these M2 macrophages resided in the outer third of the tumors. To further confirm the shift in tumor macrophage polarity associated with SF, we conducted unbiased quantitative proteomic characterization of TAMs membrane proteins. Indeed, across the spectrum of the proteins expressed in macrophage membrane, proteins that were previously identified as representing preferential M2 phenotype were predominantly present in the SF group. Considering the previously mentioned effects of chronically enhanced sympathetic outflow on innate immunity and TLR function, we further explored and detected significant increases in TLR4 expression in SF-exposed TAMs. Subsequent experiments in either TLR4 knock-out mice or in mice lacking one of the 2 major signaling pathways of TLR4, namely MYD88 or TRIF, showed that the SF-induced differences in tumor growth were dependent on alterations in TLR4 function, an area that will require expanded research to unravel the role of sleep in this immunological context. Similarly, aforementioned experiments have shed some insights on the potential protective role of NADPH oxidase within innate immune cells in the host recognition of cancer cells [53].

2b) IH, inflammation, and cancer:

Adequate oxygenation is crucial for the normal function of cells, tissues and organs, and hypoxia -a frequent occurrence in multiple pathological conditions-may result in negative outcomes. Given the importance of adequate tissue and cellular oxygenation to survival, it is not surprising that a large array of molecular pathways and cell processes are modulated by hypoxia, and that the potential deleterious effects of reduced cellular oxygen availability have been extensively studied [115,116]. Hypoxia triggers divergent generalized adaptive and maladaptive responses at different levels of severity and stimulus presentation. Modulation of variety of redox-sensitive transcriptional factors such as hypoxia-inducible factors (HIF), NF-κB/Rel family, cyclic adenosine monophosphate/protein kinaseA (cAMP/PKA)-induced transcription factors of the cAMP response element–binding protein/activating transcription factor (CREB/ATF) family, the protein kinase C-induced AP-1 family, STAT1/STAT3, Nrf2, and the mitogen-activated protein kinase transcription factor family (e.g., ELK1) have all been implicated in a vast array of cellular responses to hypoxia. Moreover, hypoxia may induce an oxidation/reduction imbalance resulting in the generation of excessive reactive oxygen species (ROS) that, being chemically very reactive, may damage or modify biomolecules such as proteins, lipids and DNA. Hypoxia also alters normal function by triggering inflammation -local and systemic -that results in increased production of a broad spectrum of cytokines [116].

Even in the absence of cardiovascular or respiratory disorders affecting tissue oxygen delivery, hypoxia can be pervasively present within tumor tissues, regardless of the

fact that the patient is well ventilated and with correct oxygenation in all his/her unaffected organs. Hypoxia in tumors is the result of the high proliferative rate of malignant cells that is not matched by a sufficiently expeditious process of angiogenesis within the tumor. The consequence is that malignant cells cannot be supplied with the amount of oxygen required by their accelerated metabolic rates. Moreover, in addition to continuous hypoxia, different regions of tumors frequently experience intermittent periods of hypoxia and normoxia resulting from the compression of vessels by abnormal growth of surrounding tumor tissue (reduction in local blood supply and thus hypoxia) or the creation of new vessels by angiogenesis (increase in local blood supply and thus re-oxygenation). Interestingly, it has been shown that some molecular responses to hypoxia are different depending on whether hypoxia occurs as a continuous or intermittent event. For instance, HIF-1 and HIF-2 are both up-regulated in continuous hypoxia; in contrast, in a situation of cyclic hypoxia-re-oxygenation, HIF-1 is up-regulated while HIF-2 may be down-regulated [116,117]. Accordingly, neoplastic processes (tumorigenesis, tumor growth and metastasis) are modulated by mechanisms regulated by both continuous and IH, as recently reviewed by Nanduri and Prabhakar [118]. For more extensive coverage of the link between hypoxia and the immune system in the context of cancer, the reader is referred to several recent reviews on this topic [119–123].

Patients with sleep breathing disturbances, and particularly those with obstructive sleep apnea, experience periods of intermittent hypoxemia induced by repetitive obstructions of the upper airway during sleep. As these oxygen desaturations of arterial blood are translated into intermittent events of oxygen partial pressure at tissue level [124,125], patients with sleep apnea could be more prone to develop malignancies. First and foremost among the factors potentially driving such risk could reside in the increased tumorigenesis caused by oxidative stress [126]. In fact, increased markers of DNA oxidation and damage have been found in patients with OSAS [127,128]. Furthermore, an oxidative stress challenge inducing DNA oxidation —concomitant with a inflammatory background in sleep apnea [129] could increase the mutation probability rate and hence increase cell malignant transformation potential. Secondly, as it has been well documented [118,130], mechanisms boosting tumor growth and metastasis are enhanced by intermittent hypoxia. Lastly, intermittent hypoxia might increase the ability of tumor cells to metastasize to other organs [131–134]. Other processes could however be also involved in the now rather compelling evidence that IH promotes tumor aggressiveness, namely the shedding of circulating DNA from tumors, particularly in mice exposed to IH [135], a finding that may have major implications for oncogenic transformation. In addition, the interactions between adipose tissues surrounding the tumor and the tumor itself could markedly alter the polarity of resident and bone-marrow recruited macrophages, and such interactions further modified by IH [136].

Most of the data currently available in the literature on how IH affects cancer progression in animal and cell models has been focused on mimicking the changes in oxygenation during tumor growth and the uneven process of vascularization, which exhibit cycling times ranging from several minutes to hours [130]. These hypoxia/re-oxygenation rates are at least one order of magnitude slower than those experienced by patients with sleep breathing disturbances. Indeed, obstructive sleep apnea is considered only moderate when the apnea-hypopnea index (AHI) is between 15 and 30 events/h and it is not unusual that patients with severe OSAS will present with a number of 60 events/h (which corresponds to hypoxia/re-oxygenation cycles of 1 min) or even more. Given that some molecular mechanisms triggered by intermittent hypoxia could depend on the frequency of the IH exposures [137], studying how cancer is modulated by hypoxia/re-oxygenation in sleep breathing disturbances is an issue still open for research, and will require carefully delineated experimental models setting realistic rates of IH [138].

3) IH and cancer:

The first question to investigate when interrogating animal models for the potential effect of IH on cancer is tumorigenesis. The specific question is whether fast hypoxia/re-oxygenation mimicking sleep apnea can directly or indirectly transform normal cells into malignant cells that would establish a *de novo* tumor. Unfortunately, the information currently available is scarce and inconclusive. Karoor et al. [139] injected chemical carcinogenic agents in mice, and observed the appearance of lung tumors when the animals were under normoxia, continuous hypoxia and IH. Tumors were enhanced in continuous hypoxia (11% O_2), but no changes were detected in the mice subjected to cycles of 2 min of normoxia and 2 min of 10% O_2 (12 h/d for 21 wk). More recently, Zhang et al. [140] have reported that subjecting rats to 60 hypoxic events/h modifies the levels of brain derived neurotrophic factor and miR-34a, which could be viewed as reflecting indirect markers of tumorigenesis. However, there are no data specifically documenting whether IH at rates relevant for sleep breathing disturbances actually enhances tumorigenesis. Future studies should investigate whether IH in conventional animal models of chemical- or radiation-induced tumorigenesis or, alternatively, investigate the appearance of spontaneous tumors in rodent strains particularly prone to development of neoplastic processes.

Whether IH increases the growth rate of already existing tumors -regardless of the origin of the malignant cells-is a second important question to investigate. Rofstad et al. [141] induced intradermal A-07 human melanoma xenografts in mice and subjected the animals to 12 cycles of 10 min of 8% O_2 followed by 10 min of air for a total of 4 h. Tumors in the mice exposed to cyclic hypoxia showed increased blood perfusion, microvascular density and vascular endothelial growth factor (VEGF) expression, along with unchanged expression of interleukin-8, platelet-derived endothelial cell growth factor and basic fibroblast growth factor expression [94]. To investigate whether tumor growth under conditions realistically mimicking the patterns of IH recorded among OSAS patients, Almendros et al. [142] subjected mice to a realistic IH paradigm consisting of 10 s of hypoxia (the animals breathed air at 5% O_2) alternating with 50 s of normoxia by breathing room air for 6 h/day during the light hours. This pattern induced recurrent oxygen desaturations in arterial blood that were remarkably similar to those observed in polysomnographic recordings of patients with moderate to severe OSAS: 60 events/h with oxyhemoglobin saturation value swings ranging from normal values (>95%) down to about 70%. Before the application of IH or of continuous normoxia in control animals, murine melanoma cells were subcutaneously injected in one flank of the mice. This conventional melanoma model induces a local tumor that progressively increases in size over 2–4 wk with subsequent metastases developing and typically occurring in the lungs. The mice subjected to IH exhibited a tumor growth rate that was approximately twice as fast as that observed in normoxic controls.

Moreover, the tumors in the mice under IH were considerably more necrotic, which is a phenotypic feature commonly regarded as a signature of aggressiveness [142]. When the same experimental model was applied to obese mice, tumor growth rates in the obese normoxic animals was similar to those in the lean animals subjected to IH [143]. Moreover, application of IH to the obese mice did not further increase tumor growth rates, suggesting that in OSAS patients, who generally tend to be obese, tumor growth could be concomitantly modulated by both IH and obesity. The high correlation found in all the animals between levels of circulating VEGF and tumor growth rates ($r = 0.76$) further suggests that this pro-angiogenic mediator could play a similar role in boosting tumor growth by IH and obesity [143]. Interestingly, using a different cancer model that employs murine lung epithelial tumor cells in mice subjected to the same IH protocol we have recently observed a similarly marked (about 2-fold) increase in tumor growth [144].

Metastasis of malignant cells from a primary tumor to a secondary organ or tissue is the third process by which IH could play a deleterious role. The fact that IH is more effective than continuous hypoxia in enhancing metastasis in vivo was already documented in 2001 by Cairns et al. [145]. However, the frequency of the hypoxic regime applied in this experiment was much lower than the one typically seen in sleep apnea (12 cycles of 10-min exposure to 5–7% O_2 followed by 10 min of normoxia, once per day, 7 d/wk). Interestingly, these investigators found that after intramuscular injection of murine KHT fibrosarcoma cells, IH -but not continuous hypoxia -increased the number of spontaneous microscopic lung metastases in the mice by a factor of about 2-fold. Several years later, Rofstad et al. [139] reported that mice exposed to cyclic hypoxia showed increased incidence of pulmonary metastases (about 3-fold when compared to controls breathing room air). This effect was attributed to the potential up-regulation of hypoxia-induced VEGF-A in the primary tumors, resulting in increased angiogenesis and blood perfusion and, therefore, facilitating tumor cell intravasation and hematogenous transport into the general circulation [139]. More recently, Almendros and colleagues [146] have shown that, compared to their normoxic counterparts, mice with subcutaneous melanoma subjected to the same OSAS-like regimen of IH described above (60 events/h) experienced a considerable increase (about 6-fold) in the incidence of micrometastases to the lung. In addition to spontaneous metastases, these authors also studied the incidence of metastasis induced by injecting melanoma cells into the peripheral blood of mice [146]. With this conventional model of induced metastasis, animals typically develop macroscopic melanoma tumors into the lungs within a few weeks. In this study, mice subjected to IH exhibited more lung metastasis (approximately 1.7-fold) than controls. Even though this difference did not reach statistical significance ($p = 0.075$), this result suggests that intravasation and homing of malignant cells into a secondary organ (the two last steps of the metastatic cascade) may also be boosted by IH [146]. IH-increased tumor vascularization and necrosis, intravasation (circulating melanoma cells) and extravasation (metastatic cells into the lung) have recently been described in a similar animal model [147]. In this preliminary study, the authors investigated the potential role of matrix metalloproteinases in metastasis and found increases in MMP-7, the expression of which is regulated by HIF-1α, and also reported decreases in MMP-9 and -12, that are regulated by HIF-2α, suggesting that IH favors HIF-1α over HIF-2α in these tumors [147]. The pro-metastatic role of IH mimicking OSAS has been confirmed in another type of primary tumor derived from lung epithelial cells, reporting that the number of mice presenting invasion into the muscle adjacent to the tumor was approximately three times higher in those animals subjected to IH when compared to controls exposed to room air [144].

Although the current evidence obtained from animal models strongly suggests that fast IH enhances cancer progression -as it was found in previous research in low frequency hypoxia-re-oxygenation models -our knowledge on the mechanisms involved is still limited. Specifically, it is not clear to what extend the general mechanisms already known to modulate the hypoxic response (for instance via modulation of HIFs activity; see Fig. 1C) for boosting tumor growth or metastasis is more relevant at the cell or at a systemic level. The question is: what is the relative importance of the direct effect of IH in malignant cells (e.g., by increasing proliferation and activating the migration/invasion machinery) vis-à-vis the indirect effects exerted by soluble factors secreted by the environment of the malignant cells which is also subjected to IH (e.g., increased blood supply by tumor vascularization, contribution of soluble factors from other cells)? Unfortunately, data from cell cultures focused on studying the effects of IH *per se* in cancer cells do not provide a clear insight on the question. Miao et al. [134] found that gastric cancer cells subjected to hypoxia-re-oxygenation every 12 h had an increased ability to migrate, invade, and self-renew compared with cells cultured under normoxic conditions. Similarly, human lung cancer cells subjected to cycles of 24 h of hypoxia/reoxygenation exhibited high rates of proliferation and invasion [148]. In addition, Gupta et al. [149] found enhancement in cell invasion, migration and angiogenesis in medulloblastoma cells subjected to hypoxic challenge every 48 h. However, other authors have reported different results. Rofstad et al. [139] subjected human melanoma cells to cycles of 30 min of hypoxia and 30 min of normoxia and found no change in the lung colonization potential of the cells. Oppegard et al. [150] also found that breast cancer cells under a similar pattern of hypoxia/normoxia did not show increased migration as compared with normoxia. More recently, Almendros et al. [144] reported no proliferative change when melanoma and lung cancer cells were subjected to 30 min cycles of hypoxia/re-oxygenation. It is possible that these inconsistent results are the result of comparing different cancer cell types and IH experimental protocols since duration of events ranged from 30 min to 48 h and level of hypoxia varied from 0.1% to 5%. It would be therefore interesting that future studies address the question of how cancer cell proliferation and migration is modified by IH patterns realistically mimicking those actually experienced by tumor cells in vivo (e.g., tumor tissue oxygen pressure swings ranged from about 8 mmHg to 45 mmHg (equivalent to approximately 1%–6% O_2) in mouse melanoma [146]. However, the experimental setting to impose well controlled fast hypoxia/normoxia cycles at cell level is difficult and will require specially designed experimental systems to operate at high cycling rates or alternatively microfluidic systems that mimic the 3-dimensional temporal changes in oxygen tensions within tumors [151,152].

Besides the direct effect that IH could have in cancer cells, there are well known extracellular mechanisms modulating cancer cell behavior. In addition to increased tumor vascularization via modified expression of transcription factors such as HIFs, hypoxia can boost recruitment of macrophages and alter their polarity, all of which may promote increased tumor growth, invasion and metastasis [153]. Specifically, the increased presence of TAMs in tumors is associated with a poor prognosis [154]. A recent study from our laboratory involving the TC1 tumor model in mice and cell culture systems has confirmed not only that the presence of TAMs is integral to increased proliferation and invasion, but has also shown that shifts in TAM polarity

associated with IH do play an important role in vivo tumorigenesis [144]. Indeed, TAMs in tumors of mice exposed to IH exhibited a shift in polarity from M1 anti-tumoral to M2b pro-tumoral phenotype. When compared with tumors of mice exposed to normoxia, TAMs from IH-exposed mice showed a greater effect in increasing cancer cell migration and extravasation. Moreover, the proliferative rates of cancer cells exposed to IH either in single culture or in co-culture with macrophages increased only when macrophages were concurrently present. These data strongly suggest that changes induced by IH in TAMs could play a major role in tumor progression and cancer cell migration [144]. Accordingly, the host immunologic response would be a major determinant in modulating the negative cancer outcomes associated with OSAS. As a corollary to these preliminary mechanistic observations, IH-exposed mice increase the shedding of cirDNA into circulation, which carries epigenetic modifications that may characterize cell populations within the tumor and underlie the increased aggressive tumor behaviors [135]. Notwithstanding such considerations, murine models of IH are also fraught with alterations in sleep structure, such that delineation of the independent aspects of IH and SF might prove difficult to dissect [155].

Conclusions

SF and IH are the major noxious challenges experienced by patients with sleep apnea. Whereas the data available to date strongly suggest that patients with sleep apnea run an increased risk of suffering neoplastic processes, the specific role(s) played by IH and SF have not been clearly established. First, the data from the two large long-term retrospective cohort studies —using established databases from Wisconsin, USA, and Spain, all used a global index of nocturnal hypoxemia as a surrogate marker of IH, instead of a more direct desaturation index. Second, although nocturnal hypoxemia was the index exhibiting the best association with cancer risk, the apnea-hypopnea index also showed a considerably high association. Taking into account that this index is also an indirect quantification of arousals, and thus of sleep disruption, current data from epidemiologic studies in humans suggest that both IH and possibly SF may contribute to cancer risk in sleep apnea, but solid proof is still lacking. The use of polysomnographic variables that specifically quantify arousals would be helpful to discern the potential contributions of SF and IH. Interestingly, research in animal models provides more clear insights into these issues. Interrogating identical animals subjected to an environment that only differs in the oxygen content of the breathed air (IH versus normoxia) has provided solid evidence into the specific effects of IH on cancer—particularly in cancer progression (tumor growth and metastasis). Similarly, implementation of SF paradigms has begun to unravel the potential role of sleep disruption in cancer biology. However, it is important to recognize that these experimental settings may not be ideal because the existing IH models can not completely rule out the effect of co-occurring sleep disturbance since IH may modify sleep patterns [155]. It should also be mentioned that the results obtained in animal models may considerably depend not only on the intermittent hypoxic paradigm applied but also on the cancer susceptibility of the animal strain and on the cancer model investigated (e.g., type of malignant cell and target organ).

Notwithstanding the potential limitations of the methodological approaches employed to date, the existing evidence strongly suggests that IH and SF could play an important role in increasing cancer incidence and mortality in patients with sleep breathing disorders, most likely by promoting sympathetic outflow, immunological alterations, or angiogenesis in the host response to the tumor that ultimately result in markedly adverse tumor properties. It goes without saying that we are still in an early phase of the knowledge acquisition process and that more intensive research is required to better understand the pathophysiologic mechanisms and the clinical implications of the recently revealed association between OSAS and cancer.

Practice points

1). The connection between chronic sleep fragmentation and intermittent hypoxia, the hallmark features of OSAS, is biologically plausible, and several putative mechanisms are described.
2). The presence of OSA may ascribe an increased risk for both development of cancer and poorer cancer prognosis.
3). It remains unclear whether adequate treatment of perturbed sleep and OSAS will reduce the risks for malignancy incidence and adverse outcomes.

Research agenda

Areas for future research may include:

1). Effects of IH and SF on transcriptional regulation and their unique effects on cancer biology.
2). Identify whether the potential effects of IH and SF on tumor biological properties, oncogenesis, proliferation, invasion, extravasation, and metastasis are specific to certain types of tumors or are universally applicable.
3). To explore the role of the autonomic nervous system alterations in cancer biology.
4). To assess the mechanistic processes underlying immune deregulation induced by IH and SF and their impact on tumorigenesis.
5). Large scale population cohorts to determine the generalizability of current evidence to all cancers or to specific malignancies.
6). Multicenter studies aimed at exploring the potential beneficial effect of adequate OSAS treatment on cancer incidence and prognosis.

Conflict of interest

The authors have no conflict of interest to declare.

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