Aging Reduces Intermittent Hypoxia–induced Lung Carcinoma Growth in a Mouse Model of Sleep Apnea

To the Editor:

Intermittent hypoxia (IH) is a highly prevalent occurrence in a large variety of respiratory diseases and has been associated with a wide array of morbidities. Moreover, recent experimental and clinical studies have revealed a potential relationship between obstructive sleep apnea (OSA) and cancer incidence and mortality (1). Indeed, IH mimicking OSA increases tumor malignant properties in melanoma and lung adenocarcinoma *in vitro* and in *in vivo* murine models (2, 3), where IH-induced immune deregulation appears to play a pivotal role in IH-induced malignancy enhancement (3, 4). However, there are still several important open questions regarding the relationship between OSA and cancer, and more particularly how aging affects IH–tumor interactions. The translational relevance of age as a potential modulator of IH-facilitated malignant properties is quite obvious considering that the prevalence of cancer and OSA increases with increasing patient age, and that similarly to IH, aging downregulates the immune system (3–5). In contrast, we should also point out that the association between OSA incidence and adverse outcomes and cancer is more prominent in younger patients (<55 yr) than in older patients (6, 7), such that the effect of advanced age is unclear. Here, we report our findings from a murine model of OSA in which we tested the hypothesis that aging would reduce the protumoral effect of IH both *in vivo* and *in vitro*, and that the aging effect would be linked to altered immune responses.

After approval by the Ethics Committee of the University of Barcelona, 45 female C57Bl/6j mice (young: $n = 24$, 2 mo old; old: $n = 21$, 20 mo old) were studied. The animals were preexposed to either IH or room air (RA) for 6 hours/day for 10 days as previously described (8) and then subcutaneously injected with 10^5 Lewis lung carcinoma (LLC1) cells (American Type Culture Collection) in the right flank while continuing their corresponding exposures (8). Four weeks later, the mice were killed and tumors were excised and weighed. A portion of each tumor was digested to evaluate the abundance of tumor-associated macrophages (TAMs) and regulatory T lymphocytes by flow cytometry (FACS Canto II, BD Biosciences), followed by analysis with FlowJo software (Tree Star) as previously described (3, 4). In addition, TAMs were isolated from each tumor with magnetic beads coupled to anti-CD11b antibody (StemCell Technologies), and the effect of TAMs on the proliferative rate of naive LLC1 cells in culture was also assessed (3). All results are shown as mean ± SE. The effects of aging (young vs. old) and treatment (RA vs. IH) were assessed by two-way ANOVA.

Consistent with previous findings (2–4, 9), young mice exposed to IH exhibited an ∼55% increase ($P = 0.007$) in tumor growth relative to RA-exposed mice. However, these changes were absent in aged mice (Figure 1, left), a finding that concurs with epidemiological evidence pointing to a prominent and significant association between OSA and cancer aggressiveness exclusively among younger patients with OSA (6, 7). We also found that chronic exposures to IH induced an ∼85% increase in macrophage infiltration in the tumors of young mice (from $7.1 \times 10^6 \pm 1.1 \times 10^6$ TAMs in RA to $13.0 \times 10^6 \pm 1.7 \times 10^6$ TAMs in IH; $P = 0.015$), but not in aged mice (from $5.4 \times 10^6 \pm 0.8 \times 10^6$ TAMs in RA to $6.8 \times 10^6 \pm 1.2 \times 10^6$ TAMs in IH; $P = 0.288$). Furthermore, the capability of TAMs to increase tumor proliferation *in vitro* was enhanced only in TAMs isolated from tumors of young mice exposed to IH (Figure 1, right). The reduced recruitment of TAMs to the tumors of old mice under IH conditions suggests that aged animals may not be as able as young mice to develop a depot of inflammatory cells with peritumoral adipose tissues (4). Moreover, it is likely that the lack of changes in the proliferative properties of naive tumor cells when placed in coculture with TAMs isolated from aged mice exposed to IH reflects the tumor microenvironment's inability to shift the polarity of TAMs to a more protumoral phenotype in aged mice, as was previously reported for young mice (3, 4). Of note, the relative abundance of regulatory T lymphocytes (expressed as the ratio to total lymphocyte [$CD3^+$ cells] counts) increased ($P = 0.042$) within the tumors of young and old mice exposed to IH (0.68% ± 0.17% vs. 0.43% ± 0.10%, respectively) compared with RA-exposed mice (0.37% ± 0.08% vs. 0.18% ± 0.06%, respectively), but were significantly reduced by aging ($P = 0.012$). These lymphocytes are potent immunosuppressive cells and have been associated with poor prognosis in many solid tumors.

Taken together, our findings suggest that IH potentiates tumor progression through changes in the immune system in young mice, and that such changes are conspicuously absent in aged mice. Given that we previously found that hypoxic severity (measured as blood oxygen desaturation) in response to obstructive apneas was remarkably similar in young and old rats (10), we postulated that the different immune responses in young and aged mice may be the consequence of reduced levels of reactive oxygen species generated in response to IH in older animals, as previously reported for rats exposed to obstructive apneas (10). A reduced oxidative stress level in older mice in response to IH could also help to explain our results, considering that IH-induced oxidative stress was recently suggested to play a pivotal role in lung adenocarcinoma aggressiveness (9). However, further characterization of metabolic rates, oxygen consumption, and tissue perfusion and oxygenation in tumors during exposure to IH in both young and old mice will allow us to better understand the differential effects caused by aging.

In summary, we show that chronological age emerges as an important factor to consider when studying the effects of IH in lung malignancies, and that the presence of a specific phenotypic presentation in younger ages may not necessarily become manifest later in life. As such, this study highlights the importance of employing experimental models that recapitulate with the utmost

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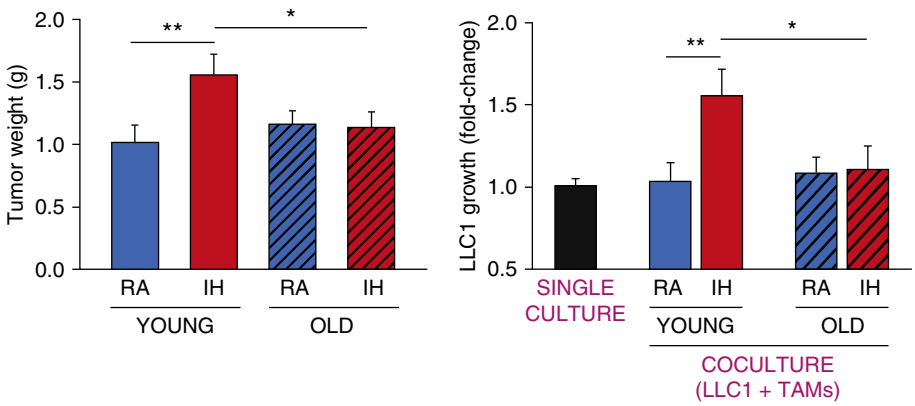

Figure 1. (Left) Tumor weight assessed in young and old mice exposed to either intermittent hypoxia (IH) or room air (RA). (Right) Proliferation of lung adenocarcinoma cells in single culture or cocultured with tumor-associated macrophages (TAMs) from young or old mice exposed to either IH or RA. Data are presented as mean ± SE. *$P < 0.05$ and **$P < 0.01$.

fidelity the pathophysiological conditions of the disease under study, in particular regarding the use of age-appropriate animals. Indeed, it is notable that most studies in animal models of chronic human diseases that primarily affect aged patients have been conducted in animals ranging in age from late adolescence to young adulthood—an issue that needs to be addressed moving forward. ■

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The Bronchial Epithelial Secretory IgA System in Asthma

To the Editor:

I read the article by Ladjemi and colleagues with great interest (1). The authors examined components of the secretory IgA system in bronchial biopsies (1), reporting less intense immunostaining for pIgR (polymeric immunoglobulin receptor) in samples from people with asthma, relative to control subjects. Reduced pIgR immunostaining in asthma appeared to be independent of global asthma severity and atopic status. In contrast, pIgR gene expression was similar in control subjects and those with asthma (1), although the authors do not comment on the inconsistency between the immunostaining and gene expression data.

To better understand the significance of these observations, readers of the *Journal* need to know if the data might be confounded by corticosteroid treatment, especially as a large proportion of the asthmatic participants were using inhaled and oral steroids. Knowing if there is a relationship between inhaled steroid dose and the secretory IgA system is of great interest, given the links between high-dose inhaled steroids and the risk of pneumonia (2, 3). The article lacks important clinical information: the basis for the assessment of asthma severity is not stated, and it is not clear how asthma control was assessed, and whether this was based on a validated questionnaire.

The authors claim in the Introduction that a prior study showed decreased IgA content in BAL fluid from patients with severe asthma (4). However, Balzar and colleagues showed that BAL fluid secretory IgA concentrations were similar in severe asthma and mild asthma, and in normal subjects (4), although serum IgA was low in severe asthma (4). Finally, Reference 12 in their article is completely unrelated to the article's subject matter and appears to have been included by mistake. I would value comments from the authors on the issues raised herein. ■

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Reply to Upham

From the Authors:

We thank Dr. Upham for his interest and comments on our article about pIgR/SC (polymeric immunoglobulin receptor/secretory component) in asthma (1). First, although our data indeed show that bronchoepithelial pIgR immunostaining is reduced in asthma, irrespective of severity or its allergic phenotype, gene expression (pIgR mRNA levels) was not significantly affected. This apparent discrepancy was also observed in chronic obstructive pulmonary disease (COPD) (2), where pIgR immunostaining was reduced in COPD and correlated with disease severity, but pIgR mRNA was upregulated in mild COPD, probably in relation to a "direct" effect of smoking (independently of COPD). An imbalance between synthesis and post-transcriptional regulation, including proteolytic cleavage (3), could explain these observations and should be investigated. Second, the potential effect of corticosteroid treatment on bronchial pIgR expression remains unknown, as already pointed

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