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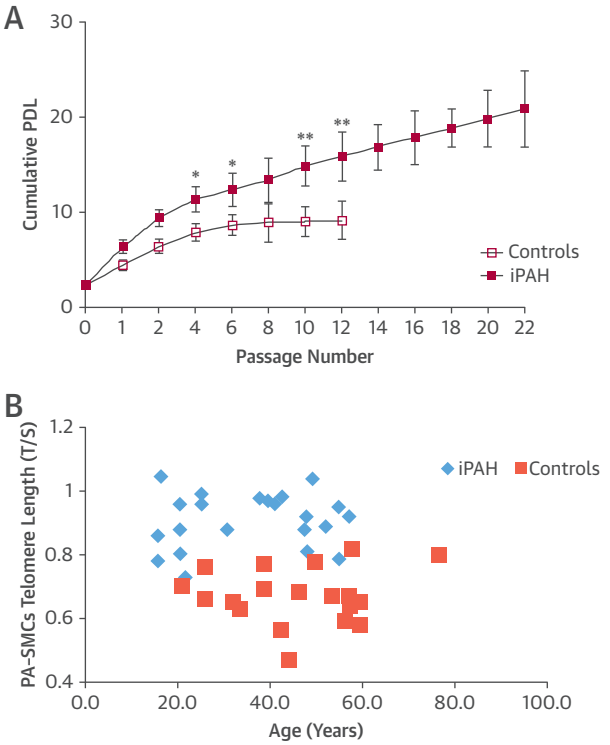
Letters

Telomere Maintenance Is a Critical Determinant in the Physiopathology of Pulmonary Hypertension

Idiopathic pulmonary arterial hypertension (iPAH) is a rare disease that occurs sporadically and in which pulmonary arterial pressure elevation leads to right heart failure and death. Although the fundamental causes remain elusive, vascular remodeling due to increased proliferation of pulmonary artery smooth muscle cells (PA-SMCs) constitutes a key feature (1,2), thus supporting the comparison between iPAH and cancer. Given the well-established link between telomere length and cell proliferation capacity, here we investigated the telomere status of PA-SMCs and its relationship to abnormal cell growth.

FIGURE 1 Growth Kinetics and Telomere Length in PA-SMCs From iPAH and Controls



(A) Growth kinetics of pulmonary artery smooth muscle cells (PA-SMCs) from controls and from patients with idiopathic pulmonary hypertension (iPAH). Cumulative population doublings were calculated at each passage by cell counting. Contrary to control SMCs, PA-SMCs keep proliferating for many more passages (n = 6 for each group, *p < 0.05 and **p < 0.01). **(B)** PA-SMCs telomere content distribution with age in patients with iPAH and in controls (n = 22 and n = 20, respectively). Telomere content in the patients' group are longer than in controls.

The local ethics committee (CPP Ile-de-France VII, Le Kremlin-Bicêtre, France) approved the study. We studied specimens from patients (informed and with consent) with iPAH and from control subjects undergoing lung transplantation or lobectomy for localized lung cancer.

For statistical analysis, all results are reported as mean ± SEM. The Mann-Whitney nonparametric test was used for comparison between groups.

In situ proliferating PA-SMCs were localized by immunostaining and counted in lung sections by using anti-proliferating-cell nuclear antigen. Then, positive cells/artery were counted, and the results were expressed as the ratios of positive cells over total cells.

Human PA-SMCs were isolated and cultured as previously described (2). Telomere length was estimated both by Southern blotting (terminal restriction fragments) or by quantitative polymerase chain reaction (3).

To assay proliferation potential, PA-SMCs were maintained in culture and were counted at each passage to determine population doubling levels.

In situ, the percent of proliferating (proliferating-cell nuclear antigen positive) PA-SMCs was significantly higher in patients with iPAH than in control subjects (data not shown). Ex vivo, as illustrated by the cumulative population doubling levels (Figure 1A), PA-SMCs from iPAH patients grew faster and for a longer time than in control subjects, indicating a higher proliferation capacity in the former. Interestingly, iPAH PA-SMCs also carried longer telomeres than control subjects (telomere repeat copy number to single gene copy number ratio [T/S] mean 0.917 vs. 0.672; Mann-Whitney test, p = 0.00084), and in contrast to control subjects, telomere length was maintained with increasing passages (data not shown).

Longer telomeres in PA-SMCs from iPAH patients did not correlate with age (**Figure 1B**). Strikingly, telomere lengths in iPAH positively and significantly (Spearman rank correlation $r^2 = 0.818$; $p = 0.00096$) correlated with pulmonary vascular resistance values, suggesting a strong relationship between disease severity and better telomere length maintenance. This phenomenon seems to be exclusive to PA-SMCs, because it was not found in matched endothelial cells or peripheral blood cells (data not shown).

Alterations in the proliferation capacity of the PA-SMCs have been associated with pulmonary hypertension. Recent studies have also highlighted the similarities between iPAH and cancer, 2 diseases that involve abnormal cell growth. Here, we extend these similarities to aspects related to telomere biology and show the correlation between telomere maintenance and proliferation capacity of iPAH PA-SMCs, as well as disease severity. This correlation, probably related to an increase of telomerase activity (4), seems to be exclusive to iPAH, because it was not found in chronic obstructive pulmonary disease pulmonary hypertension (5), suggesting that the mechanisms involved in the PA-SMC abnormal growth are different. In conclusion, our work suggests that in iPAH, PA-SMCs overcome the proliferation barriers that operate in normal cells through a better maintenance of telomeres.

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Is Caffeine Abstention Necessary Before Adenosine-Induced Fractional Flow Reserve Measurement?

Caffeine antagonizes the pharmacological actions of adenosine by blocking adenosine receptor activity (1). A protocol for adenosine stress myocardial perfusion imaging recommends that caffeine-containing products be withheld for 12 h before the test (2). However, there has been no widely accepted consensus for the need of caffeine abstention before fractional flow reserve (FFR) measurement. Conflicting results have been reported in the literature concerning the effects of caffeine on FFR measurement (3,4). Thus, we designed this study to determine if caffeine abstention is required before FFR measurement and if high-dose intracoronary adenosine overcomes the caffeine antagonism.

This prospective, single-center study enrolled 76 patients who underwent clinically indicated FFR assessment. Of these patients, 19 patients in each group were asked to refrain from caffeine-containing products for 12, 24, and 48 h before the test and 19 patients were allowed to have caffeine. Exclusion criteria included acute myocardial infarction, severe arrhythmia, previous coronary artery bypass grafting, any contraindications for adenosine or papaverine, and the presence of pressure drift (>0.03) after pull-back. Hyperemia was induced by central intravenous adenosine at a dose of 140 μg/kg/min (ADN-IV), by intracoronary adenosine at varying doses (60 μg [ADN-IC60], 150 μg [ADN-IC150], 300 μg [ADN-IC300], by 600 μg [ADN-IC600]), and by papaverine (10 to 12 mg in the right coronary artery or 15 to 20 mg in the left coronary artery), as a reference standard. The