New light in flavin autofluorescence

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Abstract

Our attention was captured by the interesting debate on the identification of lipofuscins, lipofuscin-like lipopigments, or flavins as the responsible for intracellular autofluorescence (AF) detected in epithelial cancer stem cells when exciting at 480-490 nm. Evidence was provided leading to ascribe AF emission to flavins accumulating in cytoplasmic structures, bounded to membranes and bearing ATP-dependent ABCG2 transporters. Flavins were then proposed as an intrinsic AF biomarker of cancer stem cells, with advantageous implications on cell invasiveness and chemoresistance investigations. It is however worth recalling the huge amount of literature on flavins and NAD(P)H as AF biomarkers of cell energetic metabolism and redox state, an aspect that should not be overlooked in the renewed course to extend the potential of flavins as AF biomarkers, entailing also a recent proposal of Flavin-based fluorescent proteins as substitutes of Green fluorescent proteins.

Introduction

The interesting debate on the possible contribution of lipofuscins or flavins to intracellular autofluorescence (AF) in epithelial cancer stem cells[1-3] captured our attention. The discussion making also reference to our recent review on AF in the biomedical field,[4] ended with the identification of flavins as the real primary responsible for AF signal. The almost exclusive presence of flavins was substantiated by the sole AF response to their proper excitation/emission conditions. A contribution from lipofuscins or lipofuscin-like lipopigments was excluded, since the use of wider spectral ranges in AF excitation/emission matching their broader and variable spectral properties did not result in an appreciable AF response detection.[2] In addition, reversible changes were reported for the AF signal when cells were treated with agents inhibiting ATP production. These AF changes, likely inconsistent with reversible loss of lipofuscin-like lipopigments upon administration of mitochondrial poisons, were ascribed to fluctuations of flavin AF emission. Riboflavin was therefore proposed to accumulate in cytoplasmic structures bounded to membranes and bearing ATP-dependent ABCG2 transporters, acting as an intrinsic AF biomarker of cancer stem cells with promising implications for invasiveness and chemoresistance investigation. In this concern, however, it is to remind that participation of flavins and NAD(P)H as coenzymes in redox reactions makes them to act as AF biomarker of cell redox state and engagement in energy metabolism, as from the huge amount of data reported up to now in literature.[5-13]

Autofluorescence of flavins and cell functions

In general, the AF emission from single cells and its variations depend both on the actual presence of NAD(P)H and flavins and on their redox state, considering that these endogenous fluorophores are usually the main responsible for the overall signal from single cells, and that they fluoresce in their respective reduced and oxidized state.[8,9,14,15] The redox state of the two coenzymes, in turn, depends on their strict involvement in reaction pathways of energy metabolism, reductive biosynthesis and antioxidant defense.[6,7,15-17] As reminded in our recent review on AF in cells and tissues,[4] the participation of flavins and NAD(P)H as coenzymes in intracellular reactions leading to ATP production, and the dependence of their AF emission signals on redox state inspired the pionieristic studies of Duysen, Britton Chance and coworkers on energy metabolism.[8,10,14,15,17-19] The ensuing massive works enforced step by step the importance of NAD(P)H and flavins as AF biomarkers of energy and redox state of cells and tissues.[8,20-25] The combined AF analysis of these two endogenous fluorophores was thus at the basis of remarkable experimental investigations and applications in biomedicine for the *in situ* assessment and real time monitoring of organ or cell functionality,[26-30] providing direct information on cell and tissue response ability to external stimuli or on cell intrinsic features, such as stemness degree and cancer transformation.[6,22,31-35]

Therefore, it is advisable that the role of flavins and NAD(P)H as AF biomarkers of cell energy and redox status will be not disregarded, in particular when excitation/emission conditions proper for these endogenous fluorophores are used and the effects of substances affecting mitochondrial activity are investigated. Obviously this recommendation applies in particular to *in vivo* cell investigations performed in the absence of exogenous markers, since the relatively faint native AF from mitochondria could be likely hidden by the stronger emission from fluorescent dyes used for specific organelle labelling, and consequent instrumental adjustments.

In conclusion, besides the potential of flavin and NAD(P)H AF analysis to provide information on cell energy metabolism engagement and redox state, and on their changes possibly related with adherence, tumor generation and chemoresistance, the novelty on flavins as AF biomarkers of transporters,[1] as well as on Flavin-based fluorescent proteins[36] can be expected to improve AF application leading to a more comprehensive investigation of cell functions and biological properties.

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