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Determination of the physiological state of cells by differences in FAD fluorescence intensity

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ABSTRACT

This work considers the measurement of FAD fluorescence intensity as a method for the safety, simple and real-time detection of pathological cells and informative value of this approach. FAD participates in essential processes such as fatty acid oxidation, the Krebs cycle and other redox reactions. According to literature, cells in different physiological states have different levels of FAD intensity in green-blue spectrum. Hence, it is highly relevant to determine the physiological state of cells by the difference in FAD signal intensity.

The study was realized with skin fibroblasts as a model object. On the first stage of experiments 20-days cells cultured on a pre-marked coverslips were divided into two subgroups on the basis of the autofluorescence signal intensity. The first subgroup included cells with a high autofluorescence signal (presumably senescent or pathological), and the second – cells and low one. During subsequent experiments after 24 hours necrotic cells were analyzed in a culture using Hoechst 33342 and propidium iodide in two subgroups separately.

According to the results, over 50% of cells with high autofluorescence intensity were identified as necrotic, that can subsequently be used for early diagnosis of various pathologies states. Thus, this study, with its advantages such as non-invasiveness, high sensitivity and biosafety, shows the possibility of early diagnosis of various diseases by measuring the fluorescence signal of FAD and finding cells with high fluorescence levels, which are mostly necrotic.

Keywords: FAD, autofluorescence, dead cells.

1. INTRODUCTION

Currently, optical imaging using endogenous autofluorescence of metabolic enzymes cofactors is one of the safest and most reliable ways to study the metabolic status of cells and dynamic changes in cell and tissue function both *in vitro* and *in vivo* [1-2]. This approach is characterized by its high sensitivity and ability to provide information on biochemical interactions at the molecular level. In addition, such studies offer the possibility of early diagnosis of various diseases and monitoring of response to therapeutic interventions, including real time [3-8].

The most important autofluorescent chromophores are nicotinamide adenine dinucleotide (NADH), flavinadenine dinucleotide (FAD), aromatic amino acids, and some proteins. Optical imaging using endogenous FAD fluorescence is one of the promising ways to study the metabolic status [1]. FAD participates in essential processes such as fatty acid oxidation, the Krebs cycle and other redox reactions.

$FADH_2$ is a prosthetic group of flavoproteins, which includes enzymes such as succinate dehydrogenase (tricarboxylic acid cycle and ETC complex II), MAO, xanthine oxidase, aldehydoxidase, acyl-CoA dehydrogenase. In the tricarboxylic acid cycle, succinate dehydrogenase is involved in the reversible oxidation of succinic acid to fumaric acid, which leads to the synthesis of 2 moles of adenosine triphosphate (ATP). It should be noted that in this process electrons from succinate dehydrogenase are transferred to coenzyme Q in the respiratory chain. Monoaminoxidase (MAO) is an enzyme that carries out the catabolism of monoamines through their oxidative deamination. Thus, MAO contributes to the degradation not only of endogenous monoamines (neurotransmitters and hormones) but also of exogenous ones that enter the body with food or in drugs and psychoactive substances. Xanthine oxidase, a molybdenum-containing oxidoreductase, catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid, thereby playing a central role in the breakdown of purines. Aldehydoxidase catalyzes the oxidation of various organic aldehydes and N-heterocyclic compounds to carboxylic acids, and also oxidizes quinoline and pyridine derivatives. Acyl-CoA dehydrogenase catalyzes dehydrogenation reactions from the substrate (fatty acid acyl-CoA) to the electron transferring flavoprotein (FAD) and is involved in β-oxidation. Thus, this substrate participates in such important processes as fatty

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acids oxidation, Krebs cycle and other redox reactions that explains the relevance of the study of this cofactor to determine the physiological state of cells [9]. The possibility of simple and non-invasiveness FAD determination is based on its autofluorescence, with an excitation spectrum in the wavelength range of 350-500 nm with two peaks - at 370 and 450 nm, and the emission spectrum falls in the region of 500-600 nm with a maximum at 525 nm [5].

According to literature sources, cells in different physiological states have different levels of FAD intensity in green-blue spectrum. In [10] studied different cell types, which were divided into two groups: live cells and cells treated with ethanol to induce cell death. The results showed that the viability of ethanol-treated samples did not exceed 10%. Their highest autofluorescence was observed in the green spectrum with a bandwidth between 480 and 560 nm. At the same time, the autofluorescence of the dead samples was higher compared to the live ones. Hence, it is highly relevant to determine the physiological state of cells by the difference in FAD signal intensity [5].

2. MATERIAL AND METHODS

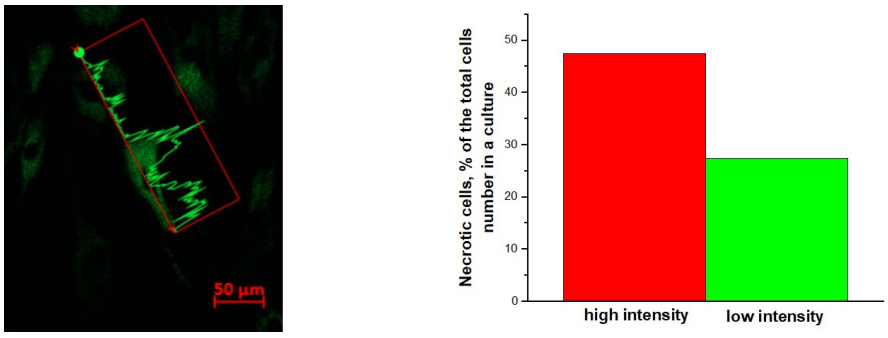
The subject of the study was a culture of skin fibroblasts. In the experiments cells were used after 20-day cultivation in DMEM-based growth medium (Gibco, UK) containing 4.5 g/l glucose, 10% fetal bovine serum (Biological Industries LDT, Israel), penicillin (100 units/ml) (Gibco, USA), streptomycin (100 μg/ml) (Gibco, USA), in a CO_2 incubator (Thermo Scientific) at 37°C, 100% relative humidity and 5% CO_2 content (Eppendorf AG). Studies were performed using a ZEISS LSM 900 laser scanning confocal microscope with Airyscan 2 system (Carl Zeiss AG, Germany) at a wavelength of 488 nm.

The first stage of the experiments was the planting of cells on glasses pre-marked with a 0.5 mm grid to locate the objects of interest. On the basis of the autofluorescence signal intensity, cells were then divided into two subgroups: those with a high autofluorescence signal (presumably senescent or pathological) and those with a low signal, the results of which were treated separately.

On the second stage after 24 hours necrotic cells were analyzed in culture using Hoechst 33342 and propidium iodide. For this purpose, cells were incubated with 5 μM Hoechst 33342 (Invitrogen, USA) (excitation/emission maxima ~ 350/481 nm) and 20 μM propidium iodide (Invitrogen, USA) (excitation/emission maxima ~ 535/617 nm) for 30 min at 37°C. Hoechst 33342 which stains nuclei of cells in different physiological state was used to count all the cells. Propidium iodide is unable to penetrate whole membranes and therefore does not stain viable cells. At the same time, damage to membrane structures occurring during necrosis, resulting in propidium iodide staining of necrotic cell nuclei.

3. EXPERIMENTAL RESULTS AND DISCUSSION

Based on results, in cell culture there were cells both high and low fluorescence intensity (Figure 1a). It could be explained by the fact that the cells had different metabolic status and were at different stages of development. In order to confirm it, the experiments described above were carried out. According to the data (Figure 1b), the proportion of necrotic cells among cells with a high FAD autofluorescence intensity was 47.4%, whereas among cells with a low autofluorescence signal – 27.3%.



a) b)

Figure 1. Research results: a – Visualization cells with high and low fluorescence signals, b – Percentage of necrotic cells of the total cell number in a culture

The findings indicate that the majority of cells with a high FAD autofluorescence intensity were necrotic. Low level viability of cells with high fluorescence in a green-blue spectrum can be a promising fact in that it enables early diagnosis of various diseases, determining the exact localization and prevalence of pathology in the tissue. Intense FAD signal can be associated with highly oxidized state of a coenzyme included in the structure of redox enzymes.

4. CONCLUSION

Thus, this study shows the possibility of early diagnosis of various diseases by detection of the fluorescence signal of FAD and finding the cells with high fluorescence intensity, which are mostly necrotic. Such approach important advantages including non-invasiveness, high sensitivity, biosafety and real-time imaging.

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