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FTIR spectral signatures of mouse antral oocytes: Molecular markers of oocyte maturation and developmental competence

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ABSTRACT

Mammalian antral oocytes with a Hoescht-positive DNA ring around the nucleolus (SN) are able to resume meiosis and to fully support the embryonic development, while oocytes with a non-surrounded nucleolus (NSN) cannot. Here, we applied FTIR microspectroscopy to characterize single SN and NSN mouse oocytes in order to try to elucidate some aspects of the mechanisms behind the different chromatin organization that impairs the full development of NSN oocyte-derived embryos. To this aim, oocytes were measured at three different stages of their maturation: just after isolation and classification as SN and NSN oocytes (time 0); after 10 h of in vitro maturation, i.e. at the completion of the metaphase I (time 1); and after 20 h of in vitro maturation, i.e. at the completion of the metaphase II (time 2). Significant spectral differences in the lipid (3050–2800 cm^{-1}) and protein (1700–1600 cm^{-1}) absorption regions were found between the two types of oocytes and among the different stages of maturation within the same oocyte type. Moreover, dramatic changes in nucleic acid content, concerning mainly the extent of transcription and polyadenylation, were detected in particular between 1000 and 800 cm^{-1}. The use of the multivariate principal component–linear discriminant analysis (PCA–LDA) enabled us to identify the maturation stage in which the separation between the two types of oocytes took place, finding as the most discriminating wavenumbers those associated to transcriptional activity and polyadenylation, in agreement with the visual analysis of the spectral data.

1. Introduction

Murine oocytes isolated from the ovarian antral compartment are characterized by two different types of chromatin organization [1,2] as in most mammalian species like the rat [3], the pig [4], the monkey [5] and the human [6]. In the Surrounded Nucleolus (SN) type, chromatin is highly condensed and forms a Hoechst positive ring around the nucleolus, while in the Not Surrounded Nucleolus (NSN) oocytes the chromatin is more dispersed and less condensed around the nucleolus [7]. The important issue of chromatin organization has been studied by several complementary techniques, such as confocal fluorescence microscopy [8] and transmission electron microscopy [6,9]. For oocytes, chromatin organization and regulation of transcription are strictly related to each other, as it is well known that heterochromatic chromatin is associated with low level of transcription. For this reason, SN oocytes are considered transcriptionally inactive while the NSN types are transcriptionally active [1,2,6]. Another important difference between SN and NSN antral oocytes concerns their ability to resume meiosis and complete, after fertilization, the embryonic development: only the SN type is able to develop till the blastocyst stage while the NSN type arrests its development at the two cell stage.

It is still unknown how the well orchestrated functional ovary originates oocytes with different destiny and which are the molecular events accounting for the two different chromatin organizations and thus two different prospective zygotic developments.

It has been suggested that the NSN-derived zygotic epigenome shows reduced levels of expression of some important genes involved

Abbreviations: A, Adenine; CPE, cytoplasmic polyadenylation element; CpG, Cytosine-phosphate-Guanine; FTIR, Fourier Transform InfraRed; GV, Germinal vesicle; GVBD, Germinal vesicle break down; MCT, Mercury cadmium telluride; MI, Metaphase I; MII, Metaphase II; mRNA, messenger RNA; NSN, not surrounded nucleolus; PCA–LDA, Principal component analysis–linear discriminant analysis; polyA, polyAdenyne; SN, Surrounded nucleolus; U, Uracil

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in cell differentiation, transcription and fatty acid oxidation [8,10]. For instance, previous experiments performed on single antral oocytes showed that the well known transcription factor Oct4 is detected with very low levels of expression in the NSN compared to the SN type: this might explain the greater developmental capacity of the SN oocytes and its correlation with the totipotent characteristic of the future zygote [10,11].

To get new insights on the SN and NSN oocyte molecular composition and organization, we used Fourier transformed infrared (FTIR) microspectroscopy, a powerful tool that allows to obtain information on complex biological systems in a non-invasive way. This technique has been widely employed in recent years to study intact cells [12–15], tissues [16–18], whole organisms [19] and to monitor *in situ* biological processes as, for instance, protein aggregation [20,21] and stem cell differentiation [13,22]. In particular, FTIR microspectroscopy allowed to identify spectral markers of putative stem cell populations in different systems, such as bovine and human cornea [23,24] and intestinal crypts [25]. Moreover, FTIR microspectroscopy has been recently employed to characterize pluripotent human embryonic and multipotent human mesenchymal stem cells, highlighting the role of lipids in the discrimination between the two different cell types [26].

In the present paper, we applied FTIR microspectroscopy to study single SN and NSN mouse oocytes at different maturation stages: antral germinal vesicle (GV), metaphase I (MI, collected from isolated SN and NSN oocytes matured for 10 h *in vitro*) and metaphase II (MII, collected from isolated SN and NSN oocytes matured for 20 h *in vitro*). Mammalian oocytes are arrested in different stages of meiotic division: during the first meiotic prophase the immature oocytes are in the GV stage characterized by a de-condensed transcriptionally active chromatin [27]. Meiotic maturation is characterized by germinal vesicle breakdown (GVBD) followed by MI and MII, which is in an arrested stage till the fertilization occurs. Taking into account the intrinsic heterogeneity of the biological samples and the complexity of their infrared absorption, we used a multivariate statistical analysis to validate and better comprehend the spectroscopic data. In particular, the use of the combined principal component analysis–linear discriminant analysis (PCA–LDA) [28,29] allowed us to recognize and pull out the most significant spectral bands that contribute to the largest spectral variance.

2. Materials and methods

2.1. Oocyte isolation and culture

For FTIR characterization, oocytes were collected at different times of their maturation - GV, MI and MII stages - to get a dynamic view of protein and nucleic acid contents. Female mice B6D2F1 (F1 CD^{-1}), purchased from Charles River (Como, Italy), were maintained at the Department Animal Facility of the University of Pavia. Six 12-week-old females were used in this study. Animals were maintained under controlled room conditions (22 °C, with 60% air moisture and 14L:10D photoperiod and fed *ad libitum*) and investigations were conducted in accordance with the guiding principles of European (n. 86/609/CEE) and Italian (n. 116/92, 8/94) laws protecting animals used for scientific research.

GV oocytes were isolated from the antral compartment and classified into SN and NSN types according to the presence or absence of a ring of Hoechst-positive chromatin surrounding the nucleolus (see Fig. 1), as already described [7], and directly used for FTIR analysis. SN and NSN oocytes were matured *in vitro* (α-mem media) [30] to collect MI (after 10 h) and MII (after 20 h) respectively.

For FTIR analysis GV, MI and MII oocytes were washed several times in a 0.9% NaCl aqueous solution to prevent medium contamination. For each cell type, single oocytes were deposited onto a BaF_2 window and dried at room temperature for about 30 min [13]. To verify that this span of time was enough to dry the samples in a reproducible way, we measured the FTIR absorption spectra of oocytes at different times of dehydration, from 30 min up to several hours. Interestingly, comparable results were found for samples dried for 30 min or longer.

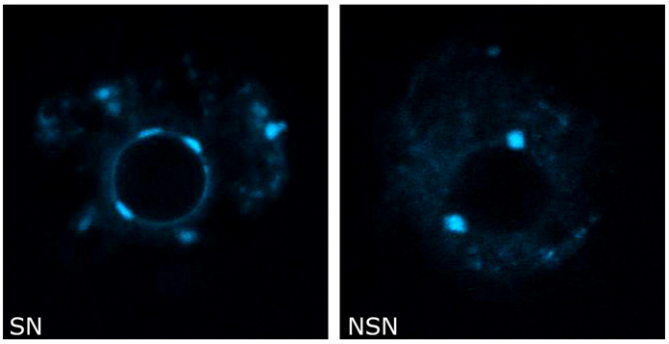

Fig. 1. Nuclei of SN and NSN antral oocytes stained with the fluorochrome Hoechst 33342. SN oocytes are characterized by a ring of Hoechst positive heterochromatin surrounding the nucleolus, that is absent in NSN oocytes. Magnification, 100×.

To evaluate spectral reproducibility, at least 10 cells/type were measured in each of the three independent experiments that we carried out.

2.2. FTIR microspectroscopy

FTIR absorption spectra of single mouse oocytes, taken at different maturation stages, were collected from 4000 to 800 cm^{-1} using a UMA 500 infrared microscope equipped with a nitrogen cooled MCT (Mercury Cadmium Telluride) detector (narrow band, 250 μm) and coupled to a FTS-40A spectrometer (both from Bio-Rad, Digilab Division, MA, USA).

Absorption spectra of single intact oocytes, with an excellent signal to noise ratio (noise of 1 mA peak to peak, all over the spectrum), were acquired in transmission mode by setting the microscope diaphragm aperture at about 100 μm × 100 μm, in order to select a whole single oocyte, whose diameter was varying from 80 to 100 μm. The spectra were collected under the following conditions: 2 cm^{-1} spectral resolution, 512 scan coadditions, 20 kHz scan speed and triangular apodization. When necessary, spectra were corrected for residual water vapor absorption.

Spectral analysis was conducted in the spectral range between 4000 and 800 cm^{-1}. To this aim, second derivative spectra were obtained following the Savitsky–Golay method (3rd grade polynomial, 11 smoothing points), after a binomial 13 smoothing points of the measured spectra, using the GRAMS/32 software (Galactic Industries Corporation, USA).

2.3. Multivariate analysis

Statistically significant spectral components were identified from the measured spectra using a combined PCA–LDA analysis [28,29], performed using MatLab R2006a (The Mathworks, USA).

The combined use of PCA and LDA allows to group large multivariate data into different clusters by maximizing the inter-cluster separation and, at same time, ensuring the minimum variability within the cluster [31,32].

The covariance matrix of the raw spectra was diagonalized to obtain the eigenvectors sorted according to the magnitude of the corresponding eigenvalue.

Only the first K eigenvectors, which describe more than the 99.9% of the total variance, were retained. In the studied systems the value of K was between 15 and 17. A set of principal components (PCA scores) was obtained projecting the original data on the subspace defined by the selected eigenvectors. The linear discriminant analysis was then