Analysis of CD36 expression with quantum dots 605 of 7-ketocholesterol-treated human monocytic cells and human atherosclerotic tissue sections by means of spectral confocal microscopy and image processing.

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The goal of this study is to evaluate the expression of CD36 with quantum dots 605 (QD 605) of untreated- and 7-ketocholesterol (7KC)-treated human monocytic U937 cells and human atherosclerotic tissue sections by confocal and multiphoton laser scanning microscopy (CLSM). Indeed, CD36 plays significant roles in atherosclerotic foam cell development. As a consequence it is of interest to develop fluorescent methods to obtain accurate identification and quantification of this receptor to improve our understanding of the mechanisms regulating its expression.

We have used U 937 cells and tissue sections and analyzed them by flow cytometry (FCM) and confocal and multiphoton CLSM. FCM was performed in different ultraviolet (UV) and visible excitation modes. Three-dimensional (3-D) sequences of images were obtained by spectral analysis in confocal and multiphoton CLSM, and analyzed by the FAMIS algorithm which provides factor curves and factor images. Factor images are the result of the FAMIS image processing method, which uses physical properties of fluorochromes (emission spectra and photobleaching velocities), assumes that each pixel is a mixture of different fluorescent patterns and makes a linear decomposition of the mixture to provide factor curves. Factor images are recomputed by projection on the factor curves and correspond to the different fluorescent compounds. In confocal and multiphoton CLSM analysis, preparations were screened in UV, 405nm and 760nm excitation modes respectively to optimize the possibilities of QD 605 and have benefit of nuclei counter-staining by Hoechst or DAPI and obtain the violet emission of 7KC simultaneously.

FCM and confocal and multiphoton CLSM reveal the expression of CD36 by means of QD 605. Factor curves and images characterize the specific red narrow emission of QD 605 and subsequent reliable identification and localization of CD36 is therefore obtained. Also, confocal and multiphoton CLSM provide the localization of 7KC versus Hoechst or DAPI. Factor curves and factor images characterize the emission of QD 605 versus emissions of 7KC and DAPI or Hoechst respectively and reliable expression image identification of CD36 is obtained.

Our investigation determines the usefulness of QD 605 to analyze antigenic expression. Following FCM, confocal and multiphoton CLSM and subsequent spectral analysis assess a more specific characterization of QD 605 fluorescent emission in the UV, 405nm and 760nm excitation modes and a simultaneous identification of 7KC. The method described above opens up new perspectives of a solution to enlarge the group of markers when some structures must be revealed by a UV-excitible compound.