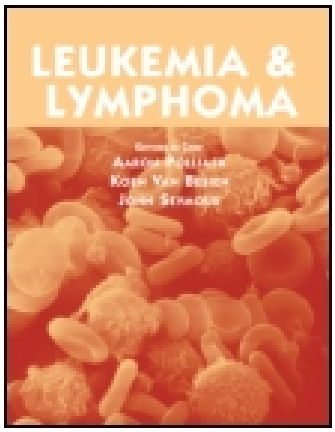

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Low-energy laser irradiation promotes cellular damage in glucocorticoid-resistant multiple myeloma cells

Alessandro Allegra[a], Enza Fazio[b], Domenico Franco[c], Marco Nicolò[c], Sebastiano Trusso[d], Fortunato Neri[b], Caterina Musolino[a] & Salvatore Guglielmino[c]

[a] Divisione di Ematologia, Dipartimento di Chirurgia Generale, Anatomia Patologica e Oncologia, University of Messina, Messina, Italy

[b] Dipartimento di Fisica e di Scienze della Terra, University of Messina, Messina, Italy

[c] Dipartimento di Scienze Biologiche e Ambientali, University of Messina, Messina, Italy

[d] CNR-Istituto per i Processi Chimico-Fisici Sede di Messina, Messina, Italy

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LETTER TO THE EDITOR

Low-energy laser irradiation promotes cellular damage in glucocorticoid-resistant multiple myeloma cells

Alessandro Allegra[1], Enza Fazio[2], Domenico Franco[3], Marco Nicolò[3], Sebastiano Trusso[4], Fortunato Neri[2], Caterina Musolino[1] & Salvatore Guglielmino[3]

[1]*Divisione di Ematologia, Dipartimento di Chirurgia Generale, Anatomia Patologica e Oncologia,* [2]*Dipartimento di Fisica e di Scienze della Terra and* [3]*Dipartimento di Scienze Biologiche e Ambientali, University of Messina, Messina, Italy and* [4]*CNR-Istituto per i Processi Chimico-Fisici Sede di Messina, Messina, Italy*

Multiple myeloma (MM) is a fatal disease characterized by the malignant proliferation of plasma cells and overproduction of monoclonal immunoglobulin or light-chain proteins. Glucocorticoids (GCs) are among the most used agents, acting through the GC receptor to induce programmed cell death. However, some patients do not respond spontaneously, or later on develop resistance to this therapy, and this has limited its application.

The mechanisms mediating this GC resistance are multifactorial. They include homologous down-regulation of the GC receptor, expression of the β isoform of this receptor, acting as a dominant negative inhibitor, or transrepression of the activated receptor by nuclear factor-κB (NF-κB). Furthermore, external stimuli as cytokines, growth factors or the bone marrow microenvironment, including extracellular matrix components and stromal cells, could confer GC resistance in MM cells [1].

MM1.S and MM1.R cells have been established as a good model to explore mechanisms of resistance to dexamethasone [2]. The MM.1 cells have typical myeloma cell morphology. Through immunocytochemical staining, it was observed that MM.1 cells contain cytoplasmic λ and not κ light chain. They express the plasma cell surface marker CD38, and human leukocyte antigen (HLA)-DR, CD59 and CD25. In the MM.1 cell line, neither translocations resulting in cyclin D1 or FGFR3 overexpression nor the dysregulation of these proteins have been observed. Together, these findings indicate that the MM.1 cell line is of plasma cell origin, and is characteristic of myeloma cells.

Since the establishment of the original MM.1 cells, sublines have been developed. A GC-sensitive cell line, MM1.S, can be distinguished by the ability of dexamethasone to inhibit cell proliferation and induce apoptosis. A resistant variant, designated MM1.R, was isolated from the original culture based on its lack of responsiveness to dexamethasone-induced cytolysis. Recent flow cytometric analysis has determined that MM1.S and MM1.R cell types express the same cell surface markers, and are immunocytochemically identical. Thus, the cell lines differ only in their sensitivity to GCs [3]. However, in the course of our studies, differences between MM1.S and MM1.R cells have been detected in Raman spectra as a variation of characteristic bands corresponding to membrane lipids and carbohydrates (data not shown).

In studies that we conducted on myeloma cells using Raman spectroscopy we were able to determine the existence of a different resistance to thermal stress of the dexamethasone-resistant myeloma cells compared to other cells.

The study was conducted on three different cell lines: MM.1S (LGC Standards, Milano, Italy), MM.1R (LGC Standards, Milano, Italy) and U266B1 (Sigma-Aldrich, Milano, Italy). Human MM cell lines were cultured in complete medium (RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine). The culture medium was bulked by centrifugation and the cells were resuspended in phosphate buffered saline (PBS). Individual round cells (5–10 cells per microscopic field) were adhered to the surface of a CaF_2 slide, previously treated with polylysine. Fixation of cells was then performed by drying or treatment with 4% paraformaldehyde for 15 min.

Raman scattering measurements were performed by means of a Horiba XploRA micro-Raman spectrometer on single cells. Since the considered cell lines show a spontaneous apoptosis level of about 4%, cells with evident alterations were excluded from the measurements. A 532 nm laser line was focused by the optics of a microscope, equipped with a × 50 and a × 100 objective, onto a 1 μm section of the sample surface. The backscattered radiation was collected by the same microscope optics and dispersed by a monochromator equipped with a 600 line/mm holographic grating, in the spectral range 400–3000 cm^{-1} with a spectral resolution

Correspondence: Alessandro Allegra, Via Consolare Valeria, 98125, University of Messina, Messina, Italy. Tel: 00390902212364. Fax: 00390902212355. E-mail: aallegra@unime.it

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of 2.0 cm^{-1}. The dispersed radiation was detected by means of a Peltier-cooled charged-coupled device (CCD) sensor. In order to avoid sample degradation, the laser power at the sample surface was kept as low as possible (< 10 mW). Accumulation times were varied, depending on the signal–noise ratio, between 3 and 50 s. All Raman spectra were normalized to their own integration times and smoothed.

We found that about 3% of dexamethasone-resistant cells showed significant alterations of spectral bands. Main differences could be linked to an alteration of characteristic peaks corresponding to symmetric and asymmetric stretching bands of nucleic acids (nucleotide conformation 600–800 cm^{-1}, backbone geometry and phosphate ion interaction, 800–1200 cm^{-1}), suggesting a structural degradation of the corresponding cell macromolecules.

Optical observation indicated that a well-defined circular hole was clearly visible in the placement of the laser beam [Figure 1(b)], confirming a minor heat stress resistance with respect to other dexamethasone-resistant cells of the sample [Figure 1(a)]. An increase of laser power or increase in exposure time did not seem to increase the damage intensity. The study was conducted in three distinct sessions on both paraformaldehyde- and dried-fixed cells. Raman spectra were obtained on at least 30 cells per session. The same damage was observed in all sessions, independent of the type of fixation employed. These results support the hypothesis that the structure of these cells was more susceptible to heat stress induced by laser irradiation.

The identification of GC-resistant MM cells remains problematic. For instance, induction of resistance to dexamethasone in the MM.1 cell line was not associated with significant variations in the cell phenotype, except for a decrease in interleukin-2 receptor (IL-2R) and HLA-DR antigen expression, in spite of the differential expression of several genes between sensitive and resistant cells. Recently, nuclear morphological alterations associated with GC resistance in human myeloma were evaluated by image cytometry. Genty *et al.* analyzed the nuclear textural phenotype of myeloma cell sub-lines resistant to GCs [4]. Their data showed a correlation between the level of nuclear abnormality observed in cell lines and the resistance index against GCs. These nuclear abnormalities in GC-resistant cell lines correspond to a progressive chromatin condensation, with large chromatin clumps heterogeneously distributed through the nucleus. Furthermore, these alterations are correlated to the expression of membrane markers associated with tumor aggressiveness [5,6].

The observation that a small percentage of cells show a decrease of Raman signals attributed to DNA may reflect

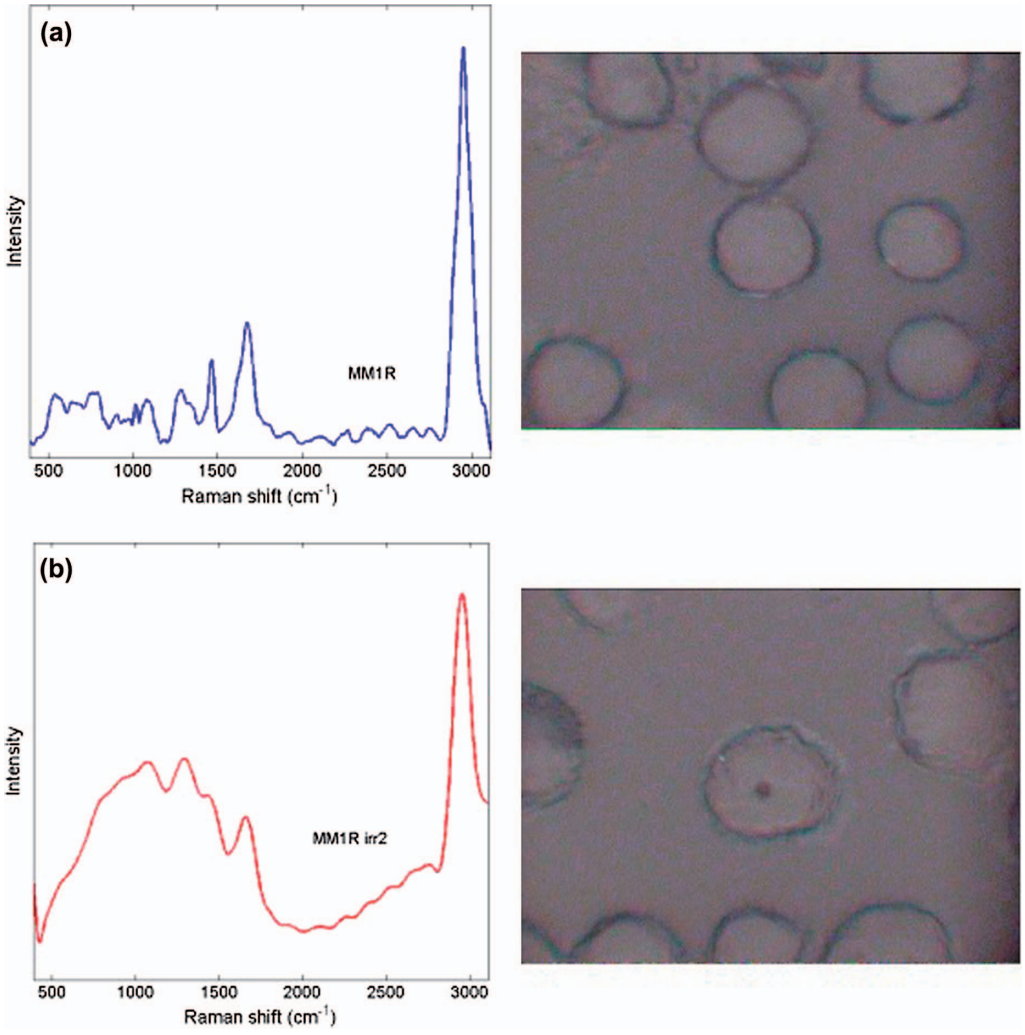

Figure 1. Raman spectra and corresponding morphologies of 97% (a) and 3% (b) MM1.R cells.

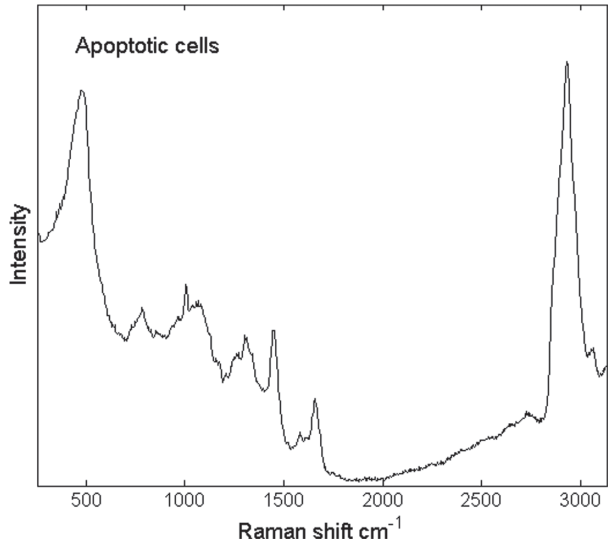

Figure 2. Raman spectrum of apoptotic cells.

some decondensation of the chromatin structure, suggesting that these cells are in intense metabolic activity. High levels of transcription, in fact, are related to decondensation of the chromatin structure.

As reported, GC-resistant cells are less susceptible to apoptosis induction, compared to GC-sensitive cells. It is likely that several factors, such as IL-6, insulin-like growth factor I, fibroblast growth factor receptor-3, Bcl2 and Hsp27, interact with either Bcl2 or cytoskeleton proteins to block dexamethasone-initiated apoptotic signaling and thereby prevent mitochondria-activated death. Moreover, restoration of sensitivity to dexamethasone causes an induction of apoptosis via the release of mitochondrial protein Smac (second mitochondria-derived activator of caspases), followed by activation of caspase-9 and caspase-3 signals [7].

On the other hand, it has been reported that Raman spectroscopy of apoptotic cells shows typical spectral variations due to reordering of the cell structure [8]. Such data are in accordance with our Raman investigations conducted on apoptotic cells (Figure 2). In fact, compared to the same non-apoptotic cells, different peaks, also found in the area related to nucleic acids, could be observed, but they were totally different from the peaks that we observed in damaged cells. Therefore, the cell fragility observed does not seem to be attributable to apoptosis, but, as already stated in the article, may instead be linked to a high metabolic and transcriptional activity of these cells.

Therefore, stress induced by laser irradiation could be responsible for the alterations observed in the peaks corresponding to the nucleic acids region, suggesting a structural degradation of such cell macromolecules. The variation of bands corresponding to membrane lipids and carbohydrates may correlate with the morphological alterations observed in these cells, and may effect the existence of a different cellular fragility.

In recent years, microirradiation with focused laser beams has emerged as a useful tool to introduce damage in live cells, and reports have shown that cell membranes readily porate, eventually leading to cell death after pulsed laser irradiation [9]. In fact, the microbeam can be directed to any subcellular or subnuclear region of interest. Damage to DNA occurs mainly via direct linear absorption. Selective induction of ultraviolet (UV) photoproducts was reported at 266 nm. At longer wavelengths (340–500 nm), side reactions are observed due to the production of reactive oxygen species in the aqueous cellular environment, which also contains endogenous sensitizers. The outcome is unwanted oxidative base modifications and DNA strand breaks. An additional mechanism that has been proposed to contribute to cell damage is low-density plasma formation [10]. On the other hand, thermal heating arising from laser irradiation is expected to play a role as a source of cell damage [11]. Additionally, heating could be accompanied by optical plasma generating shock waves with supersonic expansion and high kinetic energy. Fast bubble dynamics generate highly efficient membrane damage near the contact area [12].

The data here reported refer to effects exerted on the cells by a high-energy laser. Our data appear, for the first time, to show a reduced cell resistance of dexamethasone-resistant cells to mechanical and thermal stress induced by the laser at low or medium energy. Although the pathophysiological significance of this finding is to be explored, the different susceptibility to stress of the dexamethasone-resistant cells may be useful in assessing the mechanisms of chemoresistance and could possibly be used for therapeutic purposes.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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