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Lesioni di interesse medico-legale: espressione di marcatori miRNA nel solco cutaneo di soggetti impiccati

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Introduction

Wound age evaluation is one of the hardest challenges for the forensic pathologist when asked to establish the vitality of a skin lesion since, especially at the very beginning of the healing process, traditional histological and immunohistochemical examinations may not provide solid objective evidence. Consequently, research into the numerous biological substances involved in the process of wound repair has been carried out over the years to identify increasingly reliable biomarkers even in the very early stages of the healing process and advanced techniques have been applied to generate data with enhanced accuracy and objectivity.

Since miRNAs play a pivotal role in regulating the expression of key proteins that control the complex inflammatory response and since, after wounding, the mRNA levels of cytokines and enzymes typically change sooner than protein levels and the histomorphology, we proceeded to investigate whether the expression of some selected miRNAs was modified in ligature marks (patterned abrasion caused by ligature material) in death by hanging. At the same time, we acknowledged that gross and histological examination of these marks may sometimes be unreliable and may mislead the forensic pathologist into concluding as to whether they are due to hanging or post-mortem suspension of the body.

Aim

In this study, the expression of a panel of miRNAs was investigated in skin specimens derived from autopsy cases of death due to hanging, to clarify and to discuss their significance in assessing whether hanging marks and signs occurred before or after the death of the victim. The study was divided into two distinct phases.

In the first, skin samples taken during autopsy and then frozen immediately were analysed. In the second phase, the student tried to validate the results obtained from paraffin-embedded skin samples to verify the applicability of the miRNA investigation also to the autopsy samples routinely preserved with this method in our institutions.

To prevent possible influences in miRNA expression levels due to pathologies or diseases, all the subjects chosen were in an apparently good state of health before death. All samples were anonymized upon collection and discarded after use.

Materials and methods

Specimens (hanging marks and control skin), corresponding to skin cross-sections of 1.5 to 4.0 cm, were collected during medico-legal autopsies. A total of 39 skin samples from ligature marks and 15 samples from non-injured skin of subjects who had died by suicidal hanging were analyzed. To further assess the possible effects of degradation on miRNA profiling success, 26 skin samples from

hanging ligature marks, formalin-fixed and paraffin-embedded (FFPE) before use, were collected for the study.

Specimens were extracted using the miRNeasy Mini kit (Qiagen®) and miRNeasy FFPE kit (Qiagen®) according to the manufacturer's protocols.

Multiplexed cDNA synthesis was performed using the miScript II RT kit® (Qiagen®). cDNAs prepared in a reverse-transcription reaction served as a template for RT-PCR analysis using miScript® miRNA PCR Array - Human Cell differentiation & Development (Qiagen®). Quantification was performed using the ΔΔCt method of relative quantification and interpretation of control wells using the miScript miRNA PCR array web-based software (Qiagen®) following the manufacturer's instructions.

Results

The study showed a statistically significant increase, in term of expression, for miR-125a-5p and miR-125b-5p. Furthermore, miR-150-5p, miR-126-3p, miR-16-5p, miR-195-5p, miR-23-3p, miR-let7a-3p ($p<0.01$) and miR-let7d-3p, miR- let7c-3p, miR-let7e-3p, miR-222-3p, miR-214-3p, miR-205-5p, miR-92a-3p, miR-103a-3p ($p<0.05$) were also overexpressed.

Among these markers, miR-125a-5p and miR-125b-5p seem to be related to be related to the inflammatory response in the repair process of skin lesions. Furthermore, overexpression of additional miRNAs (miR214a-3p, miR128-3p, miR130a-3p, and miR92a-3p) with anti-inflammatory activity was highlighted. It was possible to document a statistical significance to control skin samples only for miR103a-3p ($p< 0.05$), miR214-3p and miR92a-3p ($p< 0.01$). The upregulation of miR222-3p and miR150-5p, respectively related to mast-cell activation and neutrophils after the application of traumatic stimuli supports the immunohistochemical data showed in literature.

Conclusions

The results obtained showed an increase in the expression of miRNAs recognized as regulators of the inflammatory response in skin lesions such as miR125a-5p and miR125b-5p. Furthermore, overexpression of additional miRNAs (miR214a-3p, miR128-3p, miR130a-3p, miR122-5p and miR92a-3p) with anti-inflammatory activity was highlighted; however, it was possible to document a statistical significance compared with control skin samples only for miR214a-3p, miR130a-3p and miR92a-3p.

These data confirm that miRNA expression in traumatic cutaneous insult is to be referred to an act of regulation of the inflammatory phase aimed at inhibiting the intracellular signals activated by the production of inflammatory cytokines, even in cases of lesions that develop in a very short time, of the order of a few minutes.