MicroRNAs and The Potential Use for Wound-age Estimation

The world of forensic pathology and the various technological advances used in the field are ever-evolving. More recently, microRNAs (miRNAs) are being utilized for wound-age estimation. Wound-age estimation is important for forensic pathology and can be utilized in such cases related to child, elder, and domestic abuse, as well as post-mortem injuries versus pre-mortem injuries. In general, miRNAs have a major role in regulating gene expression as well as an array of biological processes (6). Although the range of miRNA action is widespread, the following will focus on the general role of miRNAs physiologically, historical techniques of wound-age estimation, as well as the application of miRNAs in wound-age estimation.

As previously mentioned, miRNAs play a significant role in regulating gene expression and various biological processes. These processes can include signaling molecules, secretion into extracellular fluids, as well as a direct correlation between aberrant expression of miRNAs and specific pathological states. They are small non-coding RNAs transcribed from DNA sequences into primary miRNAs, precursor miRNAs, and finally mature miRNAs. The mature miRNAs then interact with the 3'UTR of target messenger RNA (mRNA) to suppress expression (6). Briefly, mRNAs are a single-stranded RNA molecule that is the compliment to a single strand of DNA. Messenger RNAs play a role in production of specific proteins (4). Therefore, when miRNAs suppress expression of the mRNA, they can suppress production of proteins that ultimately decide what biological processes occur within the body.

Wound-age determination can help forensic pathologists address the following questions:

- Was the injury caused at lifetime, in the supravital, or postmortem period?
- Is it possible to differentiate between vital and early postmortem induction?
- How long was the survival time after initial injury? (2)

Traditionally, multiple different techniques have been utilized to aid in wound-age determination. The four stages of wound-healing include hemostasis, the inflammatory stage, the proliferative stage, and remodeling (5). Routine histology with hematoxylin and eosin can be used to help identify various specific cell types associated with these 4 stages. Briefly, wound-healing starts with platelets, followed by neutrophils, macrophages, and lymphocytes in sequence. Finally, fibroblasts help with the repair process. In addition, various staining methods of paraffin-embedded blocks can also be introduced. Molecular pathology utilizes biological substances such as VEGF, TGF, and various inflammatory cytokines (7). Immunohistochemistry can be used to detect inflammatory substances that are more prominent closer to the time of death and real-time polymerase chain reaction (RT-PCR) can measure mRNA levels of inflammatory cytokines (1). Many of the previously mentioned techniques are historically used in conjunction to determine age of wounds.

In regards to wound-healing, specific miRNAs can be utilized as biochemical markers associated with a certain wound-healing stage. In the transition period from the inflammatory stage to the proliferative stage, miRNA-132 is upregulated in response to TGF-beta to inhibit leukocyte migration and stimulate growth of keratinocytes. During the proliferative phase, it is critical for keratinocytes to migrate and proliferate to induce re-epithelization and wound closure, and miRNAs play a major role in this mechanism. MiRNA-210 is just one key-player in this process, which then targets the transcription factor E2F3, which results in proliferation of keratinocytes.

During the final stage of wound-closure, miR483-3p arrests proliferation and migration of keratinocytes. An additional pathway critical in wound-healing is the AKT/mTOR pathway, which utilizes the miRNA-99 family. This set of miRNAs target and alter the expression of multiple genes within the pathway during wound-healing (6). Additionally, miRNAs are generally superior when compared to mRNA in that they are less sensitive to environmental influences, and can even be extracted with DNA to ensure adequate sample amounts (1). This factor makes their technological use in the forensic pathology field even more important to explore further.

As technology advances, it is critical that the forensic pathology community continues to discover new techniques to aid medico-legal death investigators and forensic pathologists alike in determining wound-age and as a result the exact manner of death. MicroRNAs have shown to be stable and specific enough to aid in the identification of wound-age estimation as well as additional physiological processes.

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