MicroRNAs in fracture healing and its treatment

Chapter 10

Impact

This chapter reflects on the potential scientific and societal impact of the research work described in this thesis. The aims and results of this thesis represent a valorization chain in which basic scientific work, translational large animal *in vivo* models, as well as studies focusing on the role and expression of miRNAs in the human setting have been applied. MiRNAs are made by cells, like other types of RNA, and play key roles in mRNA transcription and protein translation by targeting mRNAs or promoting the transcription of specific genes. The presented work gained important insights into the role of miRNAs in the field of trauma surgery, in particular in relation to fracture healing and multiple trauma. This thesis aimed to investigate the expression and role of miRNAs in fracture healing, putting a special focus on the influence of trauma severity, surgical fracture treatment, as well as a novel immune modulatory therapy on their expression. Thereby, this thesis also gained insights into the potential clinical translation of miRNAs to enhance bone healing and improve trauma treatment for future patients.

Relevance to the scientific and social sectors

Over the past decades, many advances have been made in the field of regenerative medicine. Among others, gene therapies and RNA therapies have gained interest over the years, the SARS-CoV-2 mRNA vaccine for the COVID-19 pandemic being an example of that advance. MiRNAs are a relatively novel type of RNA molecule which, particularly in trauma surgery, had not been researched in great detail. Over the years, it has been shown that miRNAs play vital roles in the progression and occurrence of various diseases. The work presented in this thesis shows that, also in fracture healing and trauma surgery, miRNAs are key in both healthy and impaired fracture healing and are important mediators in multiple trauma and subsequent tissue regeneration. Overall, the work in this thesis fits under the umbrella of miRNAs in fracture healing and its treatment, focusing on different aspects of trauma and trauma treatment in every chapter.

Part one of this thesis created an overview of what was known on the interplay between miRNAs and fracture healing and went on to investigate miRNA expression in the human fracture hematoma, as well as from a novel bone grafting tool. The studies in this overview identified miRNAs that are important in bone regeneration and provided insights into the involvement and role of miRNAs in both healthy and impaired fracture healing. It was shown that miRNA expression is not uniform for all patients, but also depends on specific patient characteristics and other factors, such as trauma severity. These findings are mainly important for scientists and clinicians by providing insights that are key for the translation of miRNA-based tools to the clinic, e.g. as miRNA-based therapeutics or biomarkers for the prediction of fracture healing impairments.

Another set of experiments investigated a novel surgical tool, which showed promising results in generating an autologous bone grafting material. Using this tool as a bone graft extender could reduce the requirement for alternative, more invasive autograft harvesting methods in nonunion and bone defect surgery. In addition to being of interest to scientists and clinicians, these results impact the patients suffering from impaired fracture healing, around 5 to 10% of all fractures, who

otherwise must undergo additional invasive procedures to harvest autograft, which are also accompanied by potential complications such as chronic pain.

Part two of this thesis focused on the effects of multiple trauma and different trauma surgical treatment approaches by investigating the expression of miRNAs at the fracture site as well as in the systemic circulation and determining the proteome at the fracture site. The results presented in this part of the thesis confirmed that trauma severity and injury patterns in multiple trauma go hand in hand with specific miRNA expression patterns, both at injured sites and in the systemic circulation. It was also shown that the type of surgical treatment greatly influences the local and systemic miRNAome, as well as the proteome at the fracture site. These findings are of particular interest for scientists and clinicians since, firstly, they gained insights into the role of miRNAs in multiple trauma. Secondly, they aid in clinically translating miRNAs as, e.g. diagnostic biomarkers for specific injuries such as penetrating liver injury, or as prognostic immunological markers. Third, these results elucidated biomolecular pathways by which surgical treatment influences patient recovery after trauma.

It is known that exacerbated immune responses after multiple trauma can be detrimental to a patient's overall condition, and that these immune responses can complicate clearing patients for surgery. Part three of this thesis focused on the role of the post-traumatic immune response by first investigating the expression of circulating, EV-carried miRNAs over different timepoints after trauma. EVs have gained interest over the past years due to several potential clinical applications, such as carriers of specific compounds as miRNAs, or as biomarkers for the diagnosis and/or prognosis of injury severity. The results presented in this thesis are of interest to scientists and clinicians, since they show a clear involvement of EV-carried miRNAs in multiple trauma and trauma treatment, while also shedding a light on a potential cellular communication mechanism for injury-injury crosstalk. Lastly, a novel drug was applied in a translational multiple trauma model to investigate its effect on miRNA expression at the fracture site and in the systemic circulation. This drug modulated specific parts of the innate immune system to prevent exacerbated immune activity after trauma. The results from this study showed that modulating the immune response in the acute post-traumatic phase potentially allows for earlier definitive surgery. This is of societal impact since it should enable more rapid mobilization of multiple trauma patients, and thereby contribute to reduced hospitalization times and treatment costs. Furthermore, it is of importance to scientists and clinicians since it provided insights into the effect of the novel drug, while also unveiling important biomolecular mechanisms by which trauma treatment can be guided and improved.

In short, this thesis examined and identified the expression and role of miRNAs in fracture healing in a broad and clinically relevant manner, showing that miRNA expression is key in bone regeneration, trauma, and trauma treatment. This knowledge opens doors for further research into the clinical application of miRNAs, all to aid in improving patient management and treatment after trauma. Examples of this are using miRNAs as diagnostic biomarkers for specific injuries, as

prognostic/immunological biomarkers to clear patients for surgery, and to coat/load biomaterials or bone grafting materials to enhance osteogenesis or osseointegration.

The work in this thesis is of interest to (clinical) scientists in the fields of (experimental) trauma surgery, orthopedic trauma surgeons, industrial partners, and of course the patients. The results from this thesis have been submitted and published in well-known, peer-reviewed scientific journals, such as Frontiers in Surgery, the Journal of Orthopaedic Translation, the European Journal of Trauma and Emergency Surgery, Frontiers in Immunology, and the Journal of Bone and Joint Research.

Furthermore, the results from this thesis have been disseminated to experts in the field at various distinguished scientific conferences, such as the Tissue Engineering and Regenerative Medicine International Society, the International Orthopedic Trauma Association, the European Congress of Trauma and Emergency Surgery, the biennial meeting of the International Section of Fracture Repair from the Orthopedic Research Society, and the German Congress for Orthopedic and Trauma Surgery.