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Research Methodologies In Vascular Disease

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Abstract

ARTICLES DISCUSSED:

Aomatsu, A. et al. A quantitative detection method for microRNAs in the kidney of an ischemic kidney injury mouse model. *Journal of Visualized Experiments*. (163), e61378 (2020).

Bauer, S., Pauli, J., Pelisek, J. Optimized high quality DNA extraction from formalin-fixed paraffin-embedded human atherosclerotic lesions. *Journal of Visualized Experiments*. (168), e61452 (2021).

Suárez-Cuenca, J. A. et al. Predicting amputation using local circulating mononuclear progenitor cells in angioplasty-treated patients with critical limb ischemia. Journal of Visualized Experiments. (163), e61503 (2020).

Zoli, M. et al. Role of Diffusion MRI tractography in endoscopic endonasal skull base surgery. *Journal of Visualized Experiments.* (173), e61724 (2021).

Discussion

The main purpose of the present collection is to provide the readers with up-to-date knowledge on the new methodology to study vascular disease on cell, protein, and mRNA levels, both *in vivo* and *in vitro*, in human or in a mouse model. This is meant to include new imaging and artificial intelligence modalities, as well as histopathological analysis extended to difficult tissue retrieval[1], like calcified arteries. The collection presents four methods that fully answered these questions, providing very interesting cues on how to approach old issues with a modern and technological perspective, showing a rapid technical evolution in this field.

Ischemia-reperfusion injury (IRI) is a major risk factor for acute kidney injury (AKI)[2]. No specific diagnostic biomarkers have been established, and miRNAs are excellent candidates[3]. **Aomatsu et al.**[4] developed an interesting AKI mouse model purifying and quantifying miRNAs by qRT-PCR. They identified 17 upregulated miRNAs with AKI, induced by ischemia-reperfusion damage. The method allows studying the profile of miRNAs in AKI kidneys with a dynamic range,

an accurate quantification, and a high specificity. Besides, the model facilitates the administration of drugs before and after IRI. The protocol also describes to the reader the critical points to be aware of and non-specific reactions after PCR. Some key points are the homogenization, the selection of the endogenous control, and the quality of the purified miRNAs. This method is very useful as it provides an animal model of AKI for the study of IRI in the human kidney, which is likely to be feasible for further studies beyond miRNAs.

Bauer et al.[5] set up a semi-automated method for the DNA extraction from a small amount of formalin fixed paraffin embedded (FFPE) atherosclerotic plaques. This is another interesting topic, since it is likely to provide a standardized method for studying the pathobiology of a very difficult tissue, such as diffusely calcified atheromas[1]. This makes tissue retrieval hard, as it includes the use of decalcification protocols that alter nucleic acids and other cell content[1]. The authors obtained higher DNA concentration and better DNA quality (stability and reproducibility) compared to two manual column-based methods, without significant differences in DNA fragmentation. Important protocol modifications are the incubation step for deparaffinization (less toxicity of reagents), overnight protease K digestion, further incubation time after the addition of lysis buffer at 65 °C, and increase of the elution volume. The protocol is certainly promising for future high-throughput analyses.

Suarez-Cuenca et al.[6] presented a protocol for the quantification of circulating mononuclear progenitor cells (MPCs) from vessels close to the angioplasty site, in order to predict the risk of limb amputation in patients with critical limb ischemia (CLI). This is another interesting topic, since the risk of amputation in CLI nowadays is still very high[7], and there is a substantial lack of available predictive models[8]. The possibility to identify circulating biomarkers is very promising in the clinical practice, offering a feasible perspective to change the history and the management of patients with CLI.

With the work by **Zoli et al.**[9], the imaging technique tractography was used to investigate the extension of pituitary-diencephalic and skull base tumors. Preoperative neuroimaging assessment is crucial before skull-based surgery, and tractography has been rarely adopted in skull base surgery so far due to technical issues[10]. In addition to MRI protocol implementation for the management of these tumors, the authors' goal was also to describe the translational cooperation between neurosurgeons and the neuroimaging team. This collaboration offers a multidisciplinary protocol for an effective integrated multi-axial approach to these patients. The implemented protocol included fast sequences (phase reverse encoding scans for diffusion-weighted images) as a new approach for an advanced post-processing correction. This opens the way to new approaches for the pre-surgical study of these difficult tumors.

Vascular atheromatous diseases are still a major health problem worldwide, and new methods are required more and more in order to study these patients at different levels, from imaging and blood to tissue. This collection discloses tips to overcome some issues in the study of vascular diseases, comprehending: (1) new methods to study plaque cell, protein, and mRNA content, (2) histopathological analysis extended to difficult tissue retrieval (e.g., calcified areas), and (3) the quantification of angiogenesis. Certainly, the development and divulgation of novel approaches relating to tissue analysis are pertinent to a vast range of pathologies

and potentially affect the clinical guidelines for diagnosis at all levels.

Disclosures

The authors have nothing to disclose.

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