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HMGB1 modulation in pancreatic islets using a cell-permeable A-box fragment

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ABSTRACT

Although pancreatic islet implantation is an attractive strategy for curing diabetes mellitus, implanted cells are immunologically eliminated due to early islet graft loss. One of main issues in early islet graft loss is the secretion of high-mobility group-box-1 (HMGB1) protein from the damaged islet cells, which is known as a cytokine-like factor. Therefore, regulating the activity of HMGB1 protein offers an alternative strategy for improving outcomes of islet cell therapy. To this end, we first demonstrated that HMGB1 protein could be bound to its A-box fragment (HMGB1 A-box) with higher binding affinity, resembling anti-HMGB1 antibody. To be used as a pharmaceutical protein *ex vivo*, TAT-labeled HMGB1 A-box-His_6 (TAT-HMGB1A) was structurally modified for cellular membrane penetration. TAT-HMGB1A significantly reduced secretion of endogenous HMGB1 protein through interaction in the cytosol without any damage to the viability or functionality of the islets. When TAT-HMGB1A-treated islets were implanted into diabetic nude mice, they completely cured diabetes, as evidenced by stable blood glucose level. TAT-HMGB1A treatment could also reduce the marginal islet mass needed to cure diabetes. Furthermore, TAT-HMGB1A positively protected xenotransplanted islets from xenogeneic immune reactions. Collectively, cell-penetrable TAT-HMGB1A could be used to modulate HMGB1 activity to increase successful outcomes of *ex vivo* pancreatic islet cell therapy.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronic disease characterized by destruction of insulin-secreting pancreatic islet beta cells *via* attack of autoreactive T lymphocytes [1]. T1DM has various treatments, including insulin injection, insulin pumps, and pancreas or pancreatic islet cell implantation [2]. Among these, pancreatic islet implantation has been regarded as a clinically promising therapy for restoring beta cell function because the implanted islet cells should dynamically and precisely control blood glucose levels of diabetic patients [3]. However, poor engraftment of implanted islets remains a hurdle to achieving insulin independence in diabetic recipients [4–6]. Indeed, despite improvements in islet preparation and immunosuppressive regimens, early graft loss of implanted islets requires islet masses from 2 to 3 pancreas donor organs to successfully cure a single diabetic [7]. Thus, an efficient strategy for preventing islet graft loss in the early period is needed to achieve a successful outcome. It was previously demonstrated that islet cells have high levels of HMGB1 compared with other organs, and that HMGB1 proteins are released through cytokine-induced cell damage, which acts as a trigger of the initial events of early loss of implanted islet cells [8]. Accordingly, the amount of released HMGB1 protein may reflect the degree of islet damage, acting as a prediction marker for successful outcome of islet implantation [9].

HMGB1, a non-chromosomal DNA-binding nuclear factor protein, is present in almost all eukaryotic cells [10]. Structurally, it consists of two homogenous DNA-binding domains (A- and B-box) and an acidic tail terminus. It functions to stabilize nucleosomes and regulate the transcription of numerous genes [11]. However, during cell therapy, it can be passively secreted from the cells due to cellular damage at an injection site. Outside of the cell, HMGB1 binds to receptors such as Toll-like receptors (TLRs) as well as receptors for advanced glycation end products (RAGE) of immune cells *via* its B-box fragment [12], thereby promoting maturation and migration of immune cells to the injection site of therapeutic cells [13]. In this way, HMGB1 serves as a 'cytokine-like factor' for recognition by immune cells because the passive release of HMGB1 occurs during unscheduled cell death [8]. Subsequently, HMGB1 is now recognized as a 'crucial mediator' of many diseases, including sepsis, arthritis, cancer, and diabetes [14,15], and it is very important to attenuate HMGB1 activity from injected cells. There are many therapies such as anti-HMGB1 antibodies, cytokines, anti-

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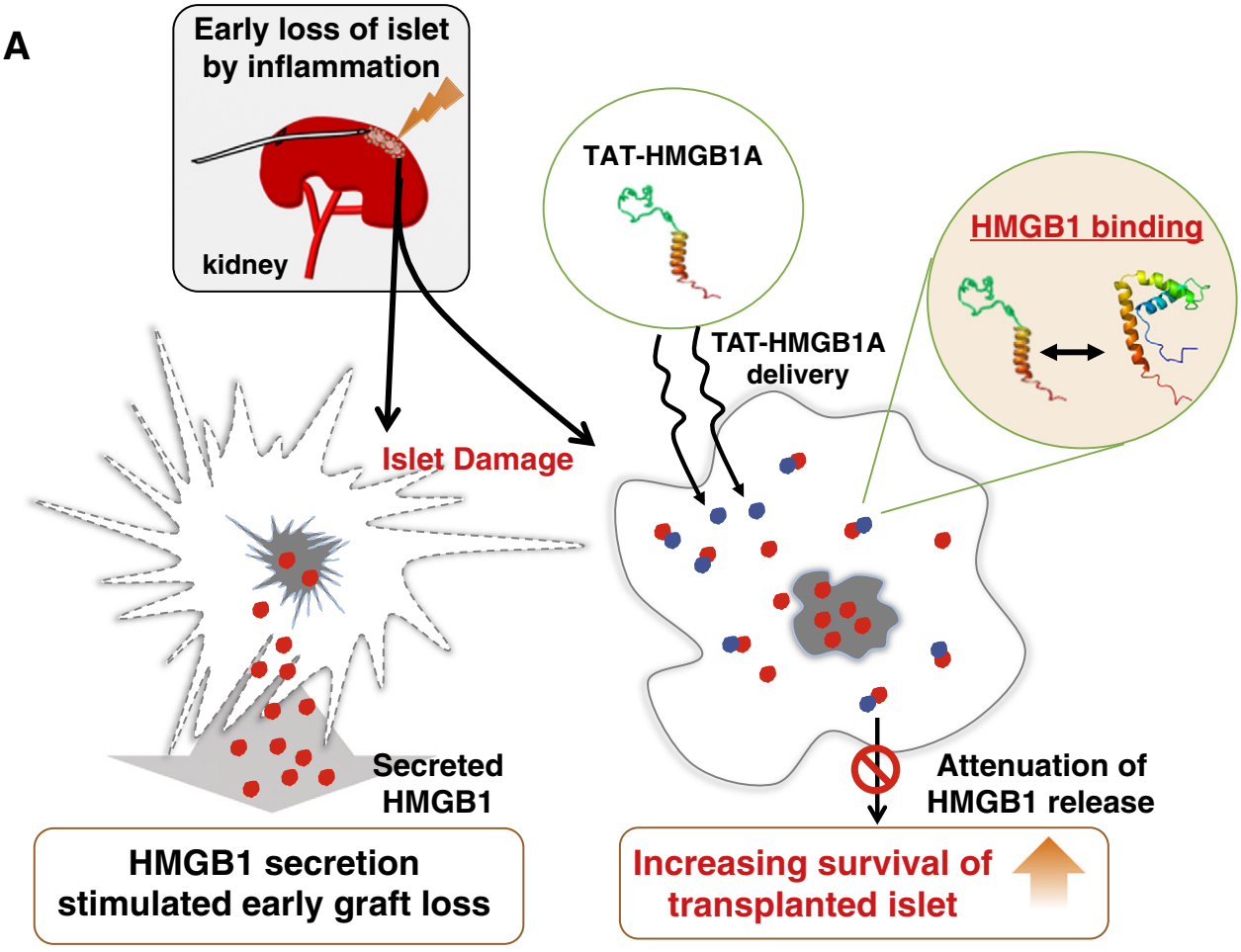

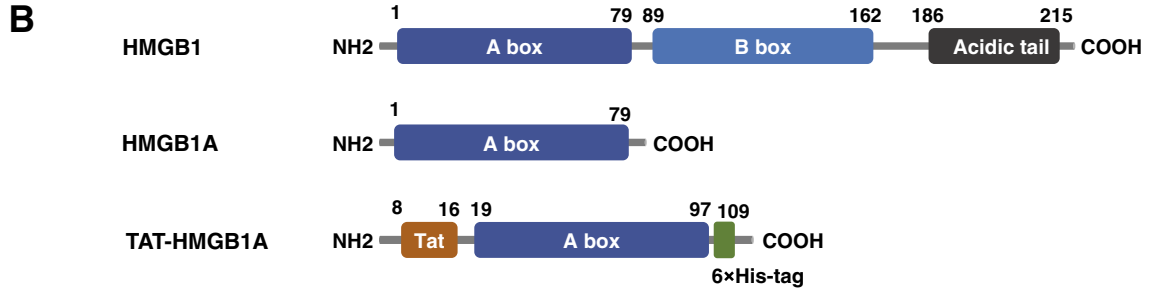

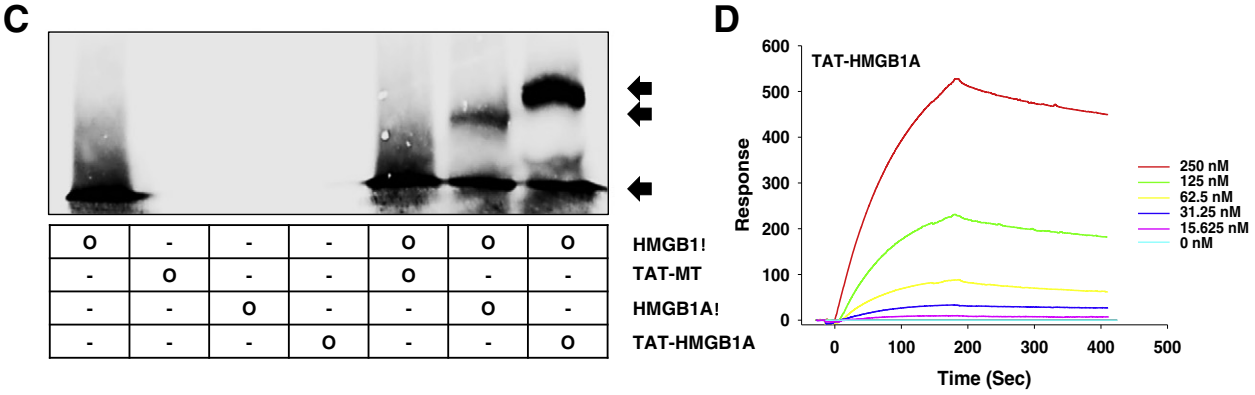

Fig. 1. (A) Scheme of TAT-HMGB1A delivery into pancreatic islet cells. TAT-HMGB1A internalization may result in binding to HMGB1, which in turn could attenuate the release of HMGB1 into the extracellular environment and reduce inflammation. This approach successfully attenuated HMGB1 release during early inflammation and damage during the early graft period through TAT-HMGB1A binding with enhancing survival of transplanted islets. (B) Simplified domain structure of HMGB1, HMGB1A, and TAT-HMGB1A. (C) Western blot assay of protein affinity *in vitro*. An anti-HMGB1 antibody was used to determine the location of HMGB1. New bands represented protein interactions with HMGB1. (D) Biacore analysis of protein interactions. HMGB1 was immobilized on a carboxymethyl dextran hydrogel surface sensor chip and the interaction with TAT-HMGB1A was measured.