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Physiology role of RAGE in bone metabolism and lung function-----
Establishing and optimisation murine soluble RAGE production in an E. coli expression system

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Promotionsfach: Innere Medizin
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Receptor for Advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules. In addition to full-length RAGE, one variant protein (C-truncated type), which is also referred as soluble RAGE (sRAGE) lacking the transmembrane and the cytosolic domain has been found which binds ligands identically to wildtype RAGE, though it does not mediate induction of cellular activation. In this study, we show the cloning and expression of murine soluble (s) RAGE in an E Coli system. RT-PCR was performed to amplify sRAGE fragment from mouse lung tissue, afterwards the msRAGE PCR products were cloned into the respective expression vector. Restriction analysis and DNA sequencing results showed that the cloning process was successful. BL21 (DE_3) containing pET-DEST42+msRAGE cells were induced with IPTG. Data showed that addition of 0.2% glucose increased the saturation density from A600 4.15 to 5.61 after 16 hours. If the sodium complex with 25mM Na_2HPO_4, 25mM KH_2PO_4, 50mM NH_4Cl, 2mM $MgSO_4$ was used in the media to increase the buffering capacity, the cells got the highest saturation density to A600 6.93 at 0.3 % glucose after 24 hours. Increasing the concentration of glycerol can also increase saturation density, However, addition of lactose in media has little effect on saturation density. Enhancement of trypton could also improve the growth of cells. At last, 0,2% glucose, 25mM Na_2HPO_4, 25mM KH_2PO_4, 50mM NH_4Cl, 2mM $MgSO_4$ were added in LB media. The cells density at A600 could reach 8 after 6.5 hours and kept steady until 24 hours in this media. Further studies showed temperature had not significant difference on msRAGE expression and IPTG turned out to be the stronger inducing factor for msRAGE expression in our system than lactose. After purification, western blot and SDS-page were performed to confirm the construction of murine sRAGE. Moreover, the recombinant

msRAGE was demonstrated to have the capacity to block S100B induced RAGE-mediated new synthesis of the NF-kB subunit p65. Thus, our studies successfully generated functional active murine soluble RAGE in an E. coli expression system, which competes with cell-bound RAGE for ligand binding and function as a “decoy” to abrogate RAGE–ligand interaction, thus providing a tool to identify the role of ligand/RAGE-interaction in specific pathophysiological settings.