Development of a fluorescent RBL reporter system for diagnosis of porcine cysticercosis

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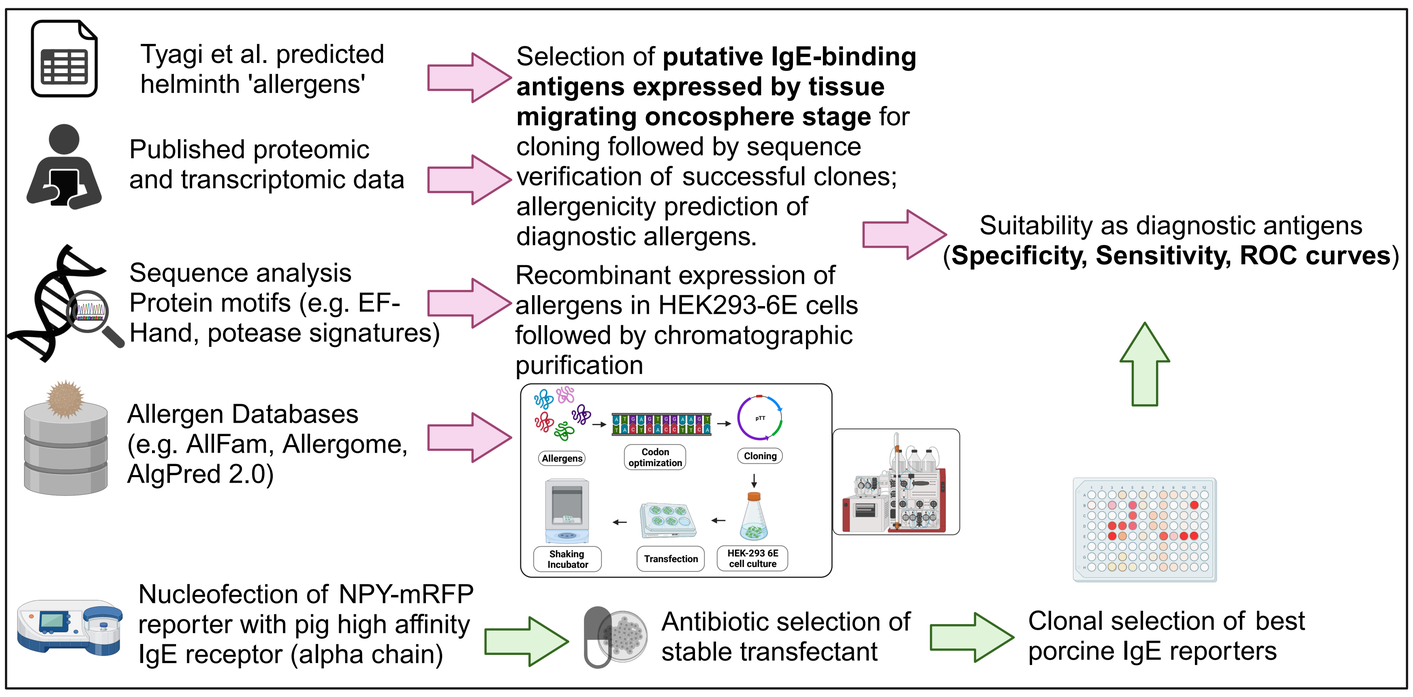
Background & Objectives

→**Porcine cysticercosis** is caused by a zoonotic neglected tropical disease parasite, *Taenia solium* in pig.
→PCC reduces pork value, affects food security and livelihood of pig farmers.
→Tongue palpation and meat inspection are most widely used diagnostic methods.
→Serological diagnosis is based on IgG, characterized by low sensitivity.
→IgE plays the central role in metazoan parasitic infections.
⊕ **Objective 1**: Development and characterization of porcinized IgE reporter cell lines which can bind pig IgE.
⊕ **Objective 2**: Selection, cloning, and recombinant expression of candidate allergens of *T. solium,* followed by their validation as diagnostic antigens.

Methods



Test Principle

⊕ Porcinized IgE reporter system created using Rat basophil leukaemia (RBL) cells stably transfected with neuropeptide Y monomeric red fluorescent protein fusion (RBL NPY-mRFP), located in granules (Fig.1).

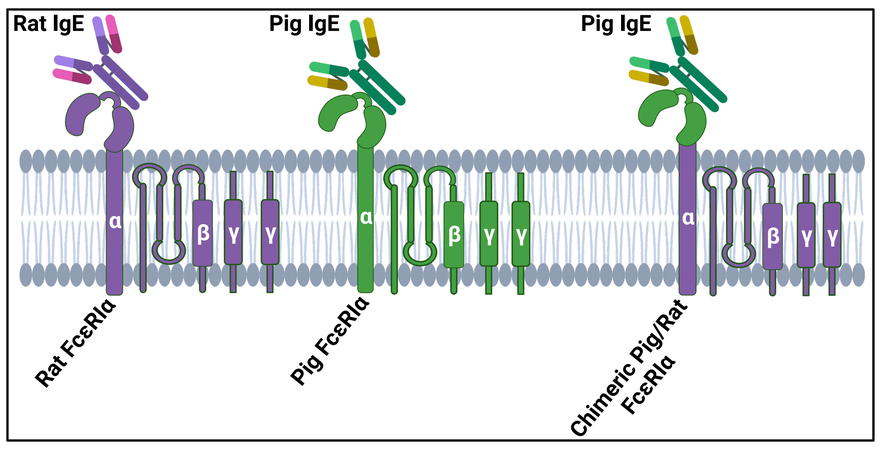


Fig. 1 Creation of chimeric pig/rat FcεRIα cell line

⊕ Porcinized reporter system incubated overnight with pig-IgE followed by stimulation with allergens, results in IgE crosslinking, by allergens, followed by degranulation of reporter cells (Fig.2).

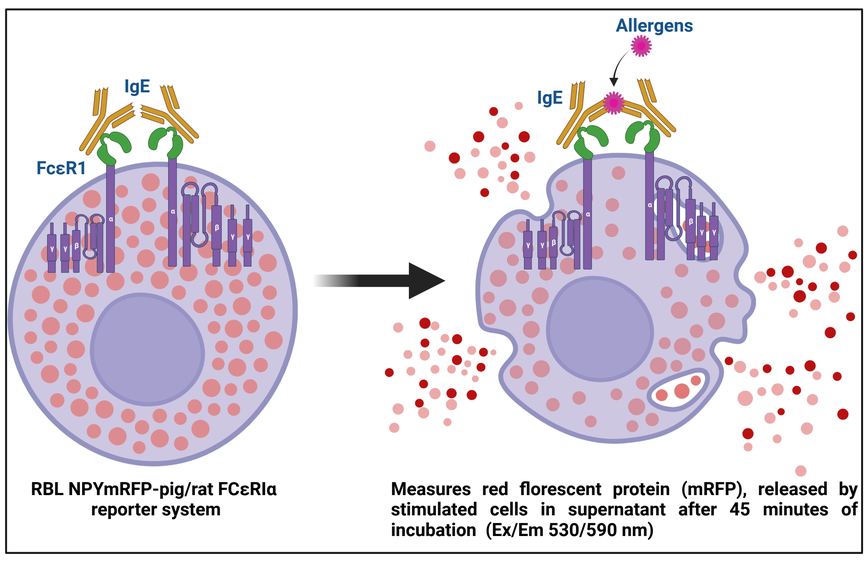


Fig.2 Activation of the porcinized IgE reporter system by IgE-allergen interaction. Crosslinking of receptor-bound IgE by allergen induces degranulation and mRFP release into supernatant.

Results

⊕ Five candidate diagnostic *T. solium* oncospheral allergens identified through bioinformatics analysis (Fig.3) and allergenicity confirmed by AlgPred 2.0.

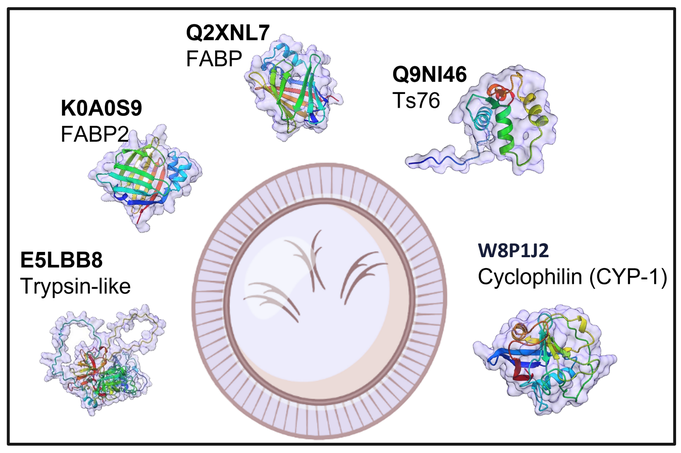


Fig. 3 Candidate allergens of *T. solium*

⊕ Three HEK293-6E cell supernatants transfected *T. solium* allergens showed expected protein band size in Western blot analysis (Fig.4).
⊕ K0A0S9: 19.8 kDa , Q2XNL7: 20 kDa, and W8P1J2: 22.3 kDa.

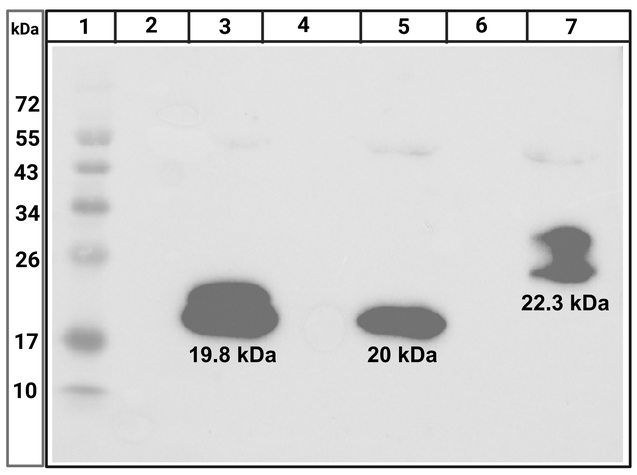


Fig. 4 Successful protein expression of transfected allergens in Western blot. Lane 1: protein ladder; Lane 2: negative control, Lane 3-7: transfected *T. solium* allergens .

Conclusion

✓ Stably transfected chimeric pig/rat FcεRIα reporter system has been created (Fig.5)
✓ Successful cloning and sequence verification of four recombinant *T. solium* allergens in expression vector
✓ Successful recombinant expression

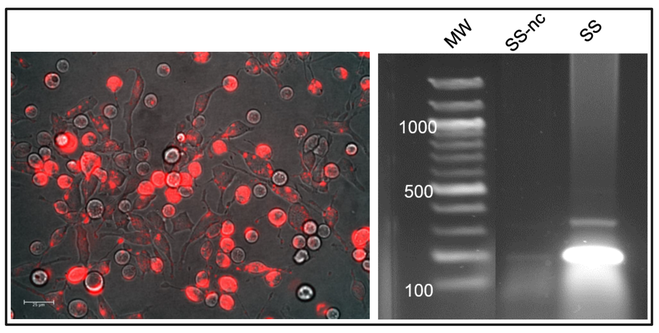


Fig. 5 Stable transfection and PCR confirmation of chimeric pig/rat FcεRIα cell line. Fluorescence microscopy showing red fluorescence from transfected cells. In PCR, RBL-2H3 cells used for negative control (SS-nc). SS: chimeric pig/ rat FcεRIα, MW is a 100 bp ladder.

Outlook

- Further expression and purification of candidate diagnostic allergens.
- Validation and assessment of Porcinized RBL reporter system with diagnostic allergens.
- Screening of infected and non-infected pig sera with reporter system.
- Determination of specificity and sensitivity.

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