Aim of the study

The aim of this study was to understand the molecular mechanisms contributing to the production of soluble leukocyte receptors in the eukaryotic expression systems including the proper posttranslational modifications such as disulfide bond formation and glycosylation. In order to achieve this goal, the following specific aims have been adopted for the study:

1. To develop new methodologies for rapid and convenient assessment of the disulfide bonds in complex eukaryotic proteins using SDS polyacrylamide electrophoresis under nonreducing conditions, and mass spectrometry.
2. To establish elements that are critical for the stability of soluble CD69 receptors expressed in the bacterial expression system.
3. To develop methods for the production of glycosylated soluble CD69 proteins in lower eukaryotes (Pichia pastoris).
4. To purify the proteins in sufficient quantities for biochemical and immunological studies.
5. To investigate, how disulfide bonding and glycosylation influences the stability of soluble CD69 receptors.
6. To look at the carbohydrate binding activities of the produced proteins.
7. To evaluate the in vivo properties of the produced proteins including their circulation half lives in the blood of experiential animals, and their efficacy in the experimental tumor therapies using the model of mouse melanoma.

In order to achieve this goals, we have adopted the suitable methodology including the bacterial and eukaryotic protein expression, biochemical techniques (chromatography, electrophoresis, analytical ultracentrifuge, protein sequences), binding assays, and immunological assays including the estimation of the antitumor efficacy.