Rubella polyvalent vaccine candidate based on chimeric hsp70 containing constructs

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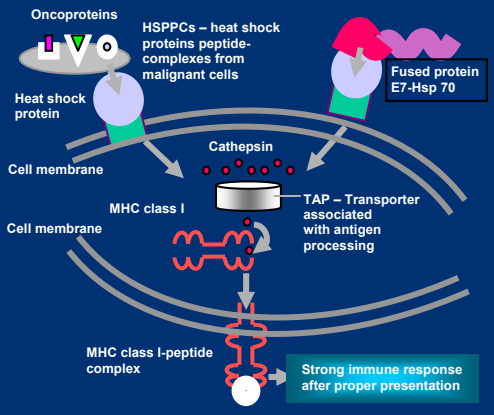
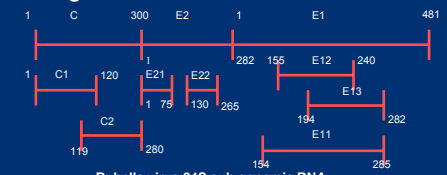
INTRODUCTION

As a prophylactic measure, vaccination against rubella is devoted mainly to the prevention of congenital deformities of the foetus (CRS) and the eradication of rubella completely. Live attenuated vaccine RA27/3 strain proved to be very effective and well tolerated. Serious concerns are arisen in connection with its application in HIV-infected people, pregnant women and newborn children. In spite of a considerable progress in cloning of structural proteins, production of virus-like particles and mapping overlapping B-and T-cell peptide epitopes, there are no new promising vaccine candidates against rubella.The goal of the present study is to evaluate an efficacy of the recombinant polyvalent vaccine based on fusion proteins with immunoadjuvant chaperone hsp70 from *M. tuberculosis*. The structure of all the constructs was His6-rubella virus polypeptide-hsp70, where rubella-specific polypeptides were 155-240aa and 194-282aa from E1, 1-75aa and 130-265aa from E2, 1-120aa and 119-280aa from core protein. To elucidate the role of hsp70 in rubella polypeptides presentation we have made fusion with full-size and two truncated variants Δhsp70(1-370aa) and Δhsp70(161-370aa). Sixteen fusion proteins were cloned into pQE30 plasmid vector, expressed in *E.coli* strain DLT1270 and purified from inclusion bodies using metallochelate affinity chromatography. Balb/c mice were immunized subcutaneously three times (0-7-21 day) with 150 μg totally of each fusion protein. The sera were tested in HIT, indirect ELISA and WB against virulent rubella virus M33 strain. Only two fusion proteins containing E12 (155-240aa) and C2 (119-280aa) fragments and full-size hsp70 induced significant seroconversion in the course of the adjuvant-free Balb/c mice immunization.

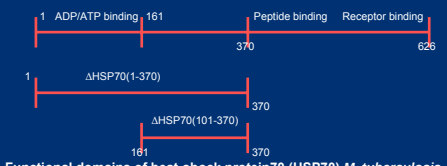
HSP 70 - based adjuvant-free immunization concept



Selection of RV genes fragments for cloning

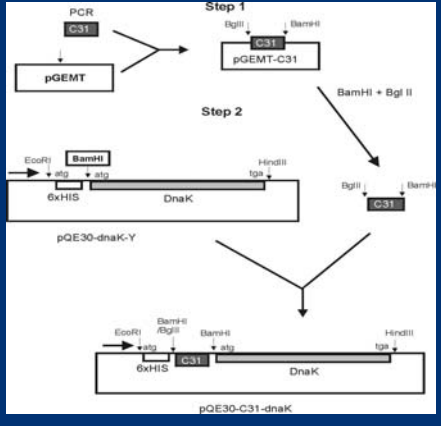


Rubella virus 24S sub-genomic RNA



Functional domains of heat-shock protein70 (HSP70) *M. tuberculosis*

Construction of plasmid pQE30-C31-dnaK



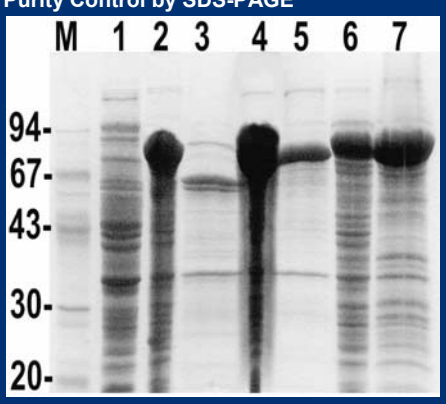
Expression of hybrid protein 6HIS-C31-DnaK in *E. coli*

1: Molecular weight markers
2: DLT1270/pQE30-C31-dnaK,uninduced
3: DLT1270/pQE30-C31-dnaK, after induction by 0.1 mM IPTG-3h

The scheme of recombinant chimeric proteins purification

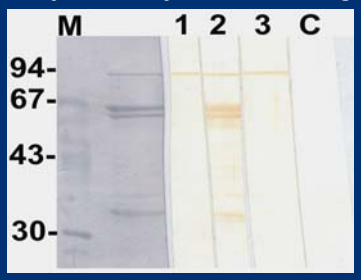
DLT1270/pQE30-RV-dnaK fermentation, 0.1 mM IPTG, 37°C, 2 h
↓
5 X wash, PBS
↓
E. Coli cell mass
↓
Ultrasonic desintegration 50 mM TRIS/HCl, 0.5 M NaCl, pH8
↓
5 X wash, 50 mM TRIS/HCl, 0.5 M NaCl, 1M urea, pH7
↓
Inclusion bodies
↓
Solubilization in 8M GuHCl, 10 mM DTT
↓
Shock Refolding and Dialysis
↓
pepRV-HSP70 chimeric proteins

Purity Control by SDS-PAGE

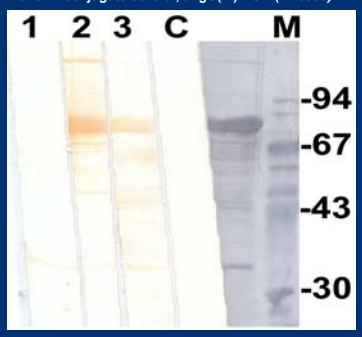


Lane M: Molecular mass standards, kDa, Lane1: DLT1270, Lane2: paste *E. coli*(HIS6-E1-3-DnaK), Lane 3: protein HIS6-E1-3-DnaK, Lane 4: paste *E. coli*(HIS6-E2-1-DnaK), Lane 5: protein HIS6-E2-1-DnaK, Lane 6: paste *E. coli*(HIS6-C2-DnaK), Lane 7: protein HIS6-C2-DnaK

Purity Control by Western Blotting



Lane M: molecular mass standards, kDa
Lane 1: MAb Ru6 (10 μg/ml, anti-E1)
Lane 2: MAb TS71 (10 μg/ml, anti-HSP70)
Lane 3: MAb HB17 (10 μg/ml, anti-His6-tag)
Lane C: conjugate control, a-IgG(m)-POD (1/20000)



Lane M: molecular mass standards, kDa
Lane 1: MAb 3D2 (10 μg/ml, anti-E2)
Lane 2: MAb TS71 (10 μg/ml, anti-HSP70)
Lane 3: MAb HB17 (10 μg/ml, anti-His6-tag)
Lane C: conjugate control, a-IgG(m)-POD (1/20000)

Rubella virus hybrid proteins properties

Name	RV Antigen	MW	pI	Expr. in *E.Coli*	Immuno-genicity	Anti-RV sera reaction	Anti-RVsero conversion
His6-E12-HSP70	E1(155-240)	77788	5.19	+	+	+	40%
His6-E13-HSP70	E1(194-282)	79165	5.22	+	+	±	-
His6-E1-ΔHSP70(1-370)	E1(154-285)	55128	5.45	+	-	-	-
His6-E1-ΔHSP78(161-370)	E1(154-285)	38208	5.44	+	+	±	-
His6-E21-HSP70	E2(1-75)	76582	5.41	+	+	±	-
His6-E21-ΔHSP70(1-370)	E2(1-75)	48933	5.78	+	+	+	-
His6-E21-ΔHSP70(161-370)	E2(1-75)	32013	5.84	+	+	-	-
His6-E22-HSP70	E2(130-265)	82237	5.41	+	+	-	-
His6-E22-ΔHSP70(1-370)	E2(130-265)	55244	6.22	+	+	-	-
His6-E22-ΔHSP70(161-370)	E2(130-265)	38324	6.44	+	+	±	-
His6-C1-HSP70	C(1-120)	n.d.	n.d.	-	n.d.	n.d.	n.d.
His6-C1-ΔHSP70(1-370)	C(1-120)	53818	7.32	±	n.d.	n.d.	n.d.
His6-C1-ΔHSP70(161-370)	C(1-120)	36898	8.81	±	-	-	-
His6-C2-HSP70	C(119-280)	85794	5.18	+	+	±	+
His6-C2-ΔHSP70(1-370)	C(119-280)	58801	5.53	+	+	+	40%
His6-C2-ΔHSP70(161-370)	C(119-280)	41881	5.55	+	+	+	100%

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