EXPRESSION AND PURIFICATION OF THE DISINTEGRIN DOMAIN OF HUMAN ADAM9 (ADAM9D)

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Members of the ADAM (A Disintegrin and Metallopeptidase) protein family are composed by a series of conserved protein domains including a disintegrin domain. The disintegrin domains of these proteins have homology with soluble snake venom disintegrins and may bind and interact with intregrins on a variety of tumor cell lines. ADAM9 is a widely expressed protein and its disintegrin domain can function as an adhesion molecule for tumor cells. The objective of this work was to express and purify the disintegrin domain of the human ADAM9 in order to study its role in the adhesion of tumor cell lines. For that, specific primers based on the human ADAM9D gene were designed (GenBank NM003816). Total RNA from VMM12 human melanoma cells was used for an RT-PCR reaction. A 270bp PCR product corresponding to the molecular mass of ADAM9D was amplified and cloned into the pGEX-4T-1 vector. The new construct (pGEX-ADAM9D) was used to transform *E. coli* AD494(DE3) cells. The synthesis of GST/ADAM9D was induced by IPTG (0.5mM, 4 hours) as confirmed by SDS-PAGE. After purification by affinity chromatography on a Glutathione Sepharose 4B resin, the ADAM9D was efficiently released from GST using thrombine protease in a resin-bound cleavage method. After purification, ADAM9D was applied in a Benzamidine Sepharose 4B resin to remove thrombine excess. ADAM9D was biologically active as confirmed by cell adhesion assays.

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