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Recombinant production and purification of the R248W mutant of human p53 in *Escherichia coli*

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The p53 protein is a tumour suppressor that specifically binds to DNA sequences to control gene expression. By controlling DNA repair, cell cycle arrest, and apoptosis, p53 prevents the dissemination of mutations within the genome. In about 50% of human cancer cases this protein is mutated. The mutation R248W in p53 (p53R248W) is designated by a contact mutation, which means that it compromises the DNA binding domain and prevents the correct activity of p53 as a transcriptional factor. This mutant promotes tumorigenesis and is related with poor prognosis and poor overall survival. In this work, the p53R248W was produced and purified in *Escherichia coli* aiming at developing novel therapeutic approaches targeting this protein. The coding sequence of the p53R248W core domain was cloned into the pETM-20 vector without any fusion patterns and expressed in E. coli BL21 (DE3). The conditions for the soluble production and purification of the recombinant protein were optimized. Some specific compounds were included in the process to improve protein stability. Yields of about 32 mg of pure p53R248W per litre of culture were obtained. The recombinant protein migrated in SDS-PAGE electrophoresis with its predicted molecular weight for a monomer (~25 kDa), which was corroborated by size-exclusion chromatography (SEC). Nonetheless, dynamic light scattering (DLS) analyses revealed high propensity for protein aggregation. Subsequent work includes an in-depth characterization of the protein at the level of its thermodynamic stability. The advances in recombinant production, purification and characterization of p53R248W to be gathered from this work will be certainly important for the progress of cancer research involving p53 proteins.

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