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Short communication

Effects of DMSO, glycerol, betaine and their combinations in detecting single nucleotide polymorphisms of epidermal growth factor receptor (EGFR) gene promoter sequence in non-small-cell lung cancer (NSCLC) patients

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ABSTRACT

The aim of the study was to examine the effects of frequently used polymerase chain reaction (PCR) additives DMSO, glycerol and betaine on amplification of GC-rich epidermal growth factor receptor (EGFR) gene promoter region, in order to detect the presence of −216G > T and −191C > A gene variations in non-small-cell lung cancer (NSCLC) patients. PCR products and restriction fragments were detected by electrophoresis on 8% polyacrylamide gel and 3% agarose gel. Our analysis shows that single used additives including DMSO in concentration of 7% and 10%, glycerol in concentration of 10%, 15% and 20%, as well as betaine in concentration of 1 M, 1.5 M and 2 M significantly enhanced the yield and specificity of PCR reaction. In addition, the combination of 10% DMSO with 15% glycerol has shown positive effects, whereas other analyzed combinations of additives failed to amplify the *EGFR* promoter region.

1. Introduction

Non-small-cell lung cancer (NSCLC), as the most frequent lung cancer type and the leading cause of cancer death worldwide [1,2], has been thoroughly researched over the years from different perspectives, but with a common aim: to improve efficiency and safety of disease treatment. Studies revealed epidermal growth factor receptor (*EGFR*) to be an important regulator of tumor growth and metastasis in NSCLC patients [3]. *EGFR* was thus recruited as a promising drug target, and two specific *EGFR* tyrosine kinase inhibitors (gefitinib and erlotinib) have been approved and widely prescribed for this indication [4,5]. However, due to specific mutations or polymorphisms in *EGFR* gene, not all NSCLC patients respond equally to this therapy [6,7]. Therefore, personalization of drug therapy in NSCLC patients requires genotyping of the *EGFR* gene, and the genotyping method needs to be reliable and easily applicable in everyday practice.

Polymerase chain reaction (PCR) is a simple genotyping technique that is used worldwide in both research and practice. Yet, it depends on a number of various parameters, which makes the optimization of the method sensitive, laborious, time and cost consuming, especially if the products should undergo further analysis, such is endonuclease cleavage in PCR-restriction fragment length polymorphism (PCR–RFLP) method [8]. Indeed, there is no single set of conditions that is optimal for all PCRs [9], especially not for DNA templates difficult to amplificate, such as GC-rich regions of *EGFR* [10]. Usual strategy in such case is adjustment of cycling conditions or components concentration, as well as utilization of additives that could enable amplification of desired target sequence [9]. The list of most important additives, reported to enhance yield and specificity of PCR, includes glycerol, betaine, formamide, dimethyl sulfoxide (DMSO), non-ionic detergents Tween 20 and Triton X-100, bovine serum albumin (BSA), and polyethylene glycol (PEG) [11–21].

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