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Targeting enteroviral 2A protease by a 16-mer synthetic peptide: Inhibition of 2Apro induced apoptosis in a stable Tet-on HeLa cell line

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ABSTRACT

Enteroviridae such as coxsackievirus are important infectious agents causing viral heart diseases. Viral protease 2A (2Apro) initiates the virus life cycle, and is an excellent target for developing antiviral drugs. Here, to evaluate the validity of the 2Apro as a proper therapeutic target, and based on the existing information and molecular dynamics, a 16-mer peptide was designed to specifically target the active site of protease 2Apro in order to block the activity of CVB3 2Apro. We showed that the peptide could compete with endogenous substrate in a concentration-dependent manner. Further, we established in HeLa cell line that expressed 2Apro. Expression of 2Apro resulted in significant morphological alteration and eventual cell death. Western blot and viability assay showed that the 16-mer peptide (200 μg/ml) could significantly block 2Apro activity and its cytotoxic effect. Future modification of the 16-mer peptide can improve its affinity for 2Apro and therefore develop effective antiviral drug.

Introduction

Coxsackievirus B3 (CVB3) is considered as the most important infectious agent that can cause severe heart complications. CVB3 is associated with both acute and chronic forms of myocarditis (inflammation of the myocardium), a life threatening disease (Reyes and Lerner, 1985; Woodruff, 1980). Chronic forms of this disease may lead to dilated cardiomyopathy (DCM), for which the only available and effective treatment is the highly cost heart transplantation procedure. Enteroviruses, in general, and CVB3, in particular, are responsible for causing up to 30–45% of acute forms of myocarditis and 25% of DCM (Bowles et al., 1985; Frisk et al., 1984). In an infected cardiomyocyte, cleavage of dystrophin by viral 2Apro is considered an important mechanism for CVB3-induced cardiac injury (Badorff et al., 1999). CVB3 infection is also associated with programmed cell death and apoptosis in myocardial tissue (Goldstaub et al., 2000; Olivetti et al., 1997).

CVB3 is a picornavirus, a family of small positive-stranded RNA viruses associated with a large variety of human and animal diseases. Following virus entry, viral RNAs are translated, and newly synthesized viral proteins are released within the cytoplasm of infected host as large a polyprotein, which will be then processed and further cleaved by virus-specific protease 2A and 3C to generate mature structural and nonstructural viral proteins (Hruskich and Wimmer, 1988; Skern and Liebig, 1994). Viral replication depends on this proteolytic cleavage. Although the newly synthesized viral polyprotein is processed mainly by 3Cpro, the primary cleavage event separating the structural protein precursor from the nonstructural one is performed by 2Apro (Hanecak et al., 1982; Toyoda et al., 1986). Viral protease 2A is a multifunctional chymotrypsin-like cysteine protease that catalyzes the cleavage of the viral polyprotein at a tyrosine-glycine pair between the C-terminus of VP1 and its own N-terminus (Rueckert, 1996; Toyoda *et* al., 1986). Both 2Apro and 3Cpro are also responsible for the cleavage of the other cellular proteins such as the eukaryotic Initiation Factor-4G (eIF4G) and the cytoplasmic protein dystrophin. Cleave of eIF4G (formerly known as p220) leads

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