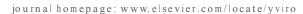
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## Virology



## 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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## A B S T R A C T

Enterovirid; ie such JS coxsackievirus are important in fectious agents causing viral heart dise; ises. Viral protease 2A (2Al'rn) initiates the virus life cycle, ;ind is an excellent target for developing Jntiviral drugs. Here, to eval u; it the valid ity of the 2AP'° as a proper the rapeutic target, and based on the existing information and moleculal dynamics, a 16-mel peptide was designed to specific; illy t;il get the active site of protease 2AP''' in order to block tile activity of CVB3 2A''''. We showed that the peptide could compete with endogenous substr; ite i n a concenti ation-dependent man ner. Further, we est, iblished ;i HeLa cell line th; it expressed 2A'''° Expression of 2AP''° resulted i n significant morphological at tera tion and eventual cell death. Western blot and viability assay showed that the 1 6-me1 peptide (200 pg/ ml ) could significantly block 2A''rn

; ictivity and its cytotoxic effect. Future modification of the 16-mer peptide c; in ill1plove its affinity for 2AP<sup>10</sup>; in d therefme develop effective a ntiviral drug.

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Introduction

Coxsackievi rus 83 (CVB3) is considered as the most important t infectious agent that can cause severe heart complications. CVB3 is associated with both acute and chronic forms of myocarditis (inflam mation of the myocardium), a life threaten ing disease (Reyes and Lerner, 1985; Woocl ruff, ±LJSO). Chronic forms of this disease may lead to dilated cardiomyopa thy (DCM), for which the only available and effective treatment is the highly cost heart transplantation proced ul e. Entroviruses, in general. and CVB3, in particular, are responsible for causing up to 30–45% of acute forms of myocarditis and 25% of DCM (Bow les et al.. 1985; Frisk et al.. 1984). In an infected cardiomyocyte, cleavage of dystrophin by viral 2AP<sup>ro</sup> is considered an important mechanism for CVB3-ind uced cardiac injury (BadoJTf et al.. 1999). CVB3 illection is also associated with program med cell death and apoptosis in myocardial tissue (Coldstaub et al., 2000; Olivetli et al., 1997).

CVB3 is a picornavirus, a family of small posi ti ve-stra nded RNA vi ruses associa ted wi th a large variety of h u man and animal diseases. Following virus entry, viral R NAs are translated. a nd newly synthesized viral proteins are released wi thin the cytoplasm of infected host as la rge a polyprotei n, which will be then processed and further cleaved by vi rus-specific protease 2A and 3C to generate ma ture structural a nd nonstrL, ctu ral viral proteins (l(ra ussl ich a nd Wi mmer. 1988; Skel n and Liebig, 1994). Viral replication depends on this proteolytic clea vage. Although the newly synthesized viral polypro-

tein is processed mainly by  $3C^{|wo|}$ , the primary cleavage event separating the structural protein precursor from the nonstructural one is performed by  $2AP^{uv}$  (l-lanecak et al., 1982; loyoda et al., 1186). Viral protease 2A is a multifunctional chymotrypsin-like cysteine proteinase that catalyzes the cleavage of the viral polyprotien at a tyrosine-glycine pair between the (-terminus of VPI and its own N-termin us (Rueckert, 1996; Toyoda *er* al., 1986). Both 2Apro a nd 3CP<sup>vo</sup> are also responsible for the cleavage of the other cellular proteins such as the eukaryotic I nitiation Factor-4G (eIF4G) and the cytoplasmic protein dystrophin. Cleave of e!F4G (formerly known as p220) leads



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