Geminin as A Molecular Target for the Development of New Anticancer Drugs

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Abstract: The unique biological properties of Geminin, particularly as an inhibitor of DNA replication initiation, have been recognized, and this has prompted a number of investigations into this molecule to explore its potential therapeutic as well as diagnostic usefulness. This review summarizes the possibility of Geminin serving as a new molecular target in the development of new anticancer drugs.

Keywords: Geminin, Cdt1, DNA replication, Cancer cell, Peptide.

INTRODUCTION

Geminin is a multifunctional protein that affects both cell division and cell differentiation [1,2]. In higher eukaryotes, Geminin has additionally evolved to regulate the activity of Cdt1, an essential regulator of DNA replication initiation. Geminin inhibits DNA replication by preventing Cdt1 from loading minichromosome maintenance (MCM) proteins onto DNA [3,4]. This highly regulated interaction between Geminin and Cdt1 prevents DNA re-replication during the same cell cycle. Besides its interaction with Cdt1, Geminin also directly interacts with Six3 and Hox proteins during embryogenesis to inhibit their functions [5,6]. The functions of Geminin of interfering with the activity of key molecules of involved in both cell proliferation and embryonic development suggests a competitive coordination of these two processes [7].

It has been observed that the loss of Geminin or overexpression of Cdt1 is associated with DNA re-replication [8]. The balance between the Geminin and Cdt1 expression levels is important for maintaining genomic integrity [9]. The imbalance in the activities of these two proteins causes the activation of key checkpoint proteins, the ATM/ATR kinases and the tumor suppressor gene, p53 [10]. In this review, we summarize the possibility of Geminin serving as a new molecular target in the development of new anticancer drugs.

GEMININ AND THE CELL CYCLE

Geminin degradation occurs at the metaphase-anaphase transition, mediated by the anaphase-promoting complex (APC), and this requires its N-terminal destruction box motif. Geminin is absent in the G1 phase when Cdt1 is present. As the S phase starts, Cdt1 is degraded and the Geminin level increases, to remain high until the completion of mitosis. In mammalian cancer cells, a high Cdt1-Geminin ratio was associated with DNA re-replication [11]. To explore the effect of non-degradable Geminin on the human cell cycle, a mutation was induced in the N-terminal destruction box of Geminin. The cells in which such stable Geminin was overexpressed showed growth arrest in the G1 phase of the cell cycle [12]. Similarly, expression of the destruction box-deleted Geminin in U2OS cells caused a cell-cycle arrest and apoptosis [13]. Endogenous levels of stable Geminin were sufficient to inhibit cellular proliferation and cell cycle arrest in HCT116 cells [14]. These experimental observations suggest that Geminin is a negative regulator of DNA replication during the cell cycle and may have a putative tumor suppressor function.

GEMININ AND CANCER

Although Geminin inhibits DNA replication and thereby, cellular proliferation, the Geminin expression levels were directly related to the cellular proliferation index in proliferating malignant cells [12,15]. Lending support to this phenomenon, it was found that increased Geminin expression is a powerful independent indicator of adverse prognosis in cases of invasive breast cancer [16]. Moreover, Geminin expression was found to be increased in more than half of the cases of colorectal cancer, as compared with the corresponding expression in the adjacent normal mucosa. A high expression level of Geminin was also detected by immunohistochemistry in 60% of human primary breast cancers [17].

STRUCTURE OF GEMININ

Geminin structure was first predicted to possess a coiled-coil domain in the central part of the protein [1]. Subsequently, the preliminary crystal structure of the coiled-coil region of Geminin was reported to be a parallel coiled-coil structure [18]. Geminin forms a dimer through its coiled-coil domain with the central region of Cdt1, which is also involved in DNA binding [19]. The Cdt1-binding domain of Geminin lies immediately adjacent to, and overlaps the dimerization domain [20]. The C-terminus of Cdt1 binds to the MCM complex. It has been shown that Geminin can inhibit the binding of Cdt1 with DNA or the MCM complex [21,22]. Recently, the molecular structure of Geminin has been determined by EM and image processing at a resolution of 17.5 Å [23]. The Geminin molecule is a tetramer formed by two dimmers, with the monomers interacting via coiled-coil domains. A rigid cylinder with negative surface charges is a critical component of the

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bipartite interaction interface between Geminin and its cellular targets [24]. Elucidation of the unusual structural organization of Geminin may provide further molecular insight for the development of unique compounds mimicking Geminin in structure and having tumor-suppressive activity.

THERAPEUTIC POTENTIAL OF GEMININ

Geminin as well as MCMs qualify exceptionally well as novel cell cycle biomarkers for routine use in clinical practice, particularly in cancer detection and estimation of prognosis [25]. Geminin inhibits the replication of a plasmid bearing the oriP replicator of Epstein Barr virus (EBV) in human cells [14,26]. Theses evidences lend support to the contention that Geminin may serve as a unique target in the field of medicinal chemistry for the development of new anticancer drugs. To identify the peptide that binds to Geminin to thereby alter the DNA replication activity in human cancer cells, we screened a phage display library of random peptides in successive cycles of phage library panning, and found one peptide sequence that bound to the 31-111 amino acid residues of Geminin. Delivery of this peptide sequence into the nucleus of HCT116 cells resulted in the suppression of BrdU incorporation into the cellular nuclei [27]. At this moment, we have no idea as to the nature of the interaction of this peptide with Geminin. However, we consider that the peptide would be an ideal starting point for the development of more effective derivatives.

SUMMARY

Despite the inconsistent experimental and clinical observations, Geminin is still promising as a drug target, because of its specific function of limiting DNA replication to once per cell cycle. Accumulating data suggest that overexpression of Geminin can kill cancer cells while leaving their counterparts unharmed. It is high time drugs designed to selectively destroy cancer cells, mimicking Geminin's effects, were developed. Geminin is very effective at killing cancer cells, but it is too large a molecule to be delivered as an anticancer drug. We are, therefore, now screening for smaller molecules that may have a similar effect as Geminin or may amplify Geminin's functions. Elucidation of the structure of Geminin may help us to develop a molecule that interferes with protein-protein interactions involving Geminin. Our recent work revealed that a 12-mer peptide binding to the central region of Geminin suppresses DNA replication activity. These results provide new insights into the functions of Geminin and further validate Geminin as a potential therapeutic target in tumor cells.

ABBREVIATIONS

MCM = Minichromosome maintenance

APC = Anaphase-promoting complex

EBV = Epstein Barr virus

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