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Methionine enkephalin, its role in immunoregulation and cancer therapy

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ABSTRACT

Methionine enkephalin (MENK), an endogenous neuropeptide has a crucial role in both neuroendocrine and immune systems. MENK is believed to have an immunoregulatory activity to have cancer biotherapy activity by binding to the opioid receptors on immune and cancer cells. Clinical trial studies in cancer patients have shown that MENK activates immune cells directly and by inhibiting regulatory T-cells (Tregs). MENK may also change the tumor microenvironment by binding to opioid receptor on or in cancer cells. All of these mechanisms of action have biologic significance and potential for use in cancer immunotherapy. Furthermore, they reveal a relationship between the endocrine and immune systems. Due to the apparent role of MENK in cancer therapy we reviewed herein, the research undertaken with MENK in recent years; which has advanced our understanding of the role MENK has in cancer progression and its relationship to immunity, supporting MENK as a new strategy for cancer immunotherapy.

1. Introduction

Methionine enkephalin (Met-enkephalin, MENK), was originally discovered by Hughes in 1975 [1]. It is an endogenous opioid, derived from pre-enkephalin [1] and has the amino acid sequence of Tyr-Gly-Gly-Phe-Met. MENK is found in the blood at low concentrations and is present in all parts of the nervous system. Studies into the neuroanatomic relationship of MENK have found higher levels of MENK in the caudate nucleus, globus pallidus, putamen and substantia nigra by radioimmunoassay. As a member of the endogenous opiate family, MENK is well-known as a long-lasting analgesic [2], and has an important role in modulating pain sensitivity.

However, following the discovery of a relationship between the endocrine and immune systems by Wybran et.al [3], the opioid receptors, like kappa, delta and mu have been detected on the membranes of immune cells including T-cells, NK-cells, macrophages, and dendritic cells. There are increasing numbers of reports supporting the observation that MENK, is involved in a regulatory loop between the neuroendocrine and immune systems, and has an immune modulatory role.

We conclude that MENK may be an immune augmenting agent with potential to restore impaired immunity by binding to one or more of the MENK receptors on immune and tumor cells. Based on this working hypothesis, we considered the use of MENK for the treatment of cancer patients, whose immune systems were damaged by tumor growth, chemotherapy or radiotherapy. The immunological effects and anticancer functions of MENK were first reported in the 1980's [4]. In recent years, with the development of neurochemical and molecular biologic techniques, especially the use of gene knockout mice, a number of reports have shown that MENK, in a dose dependent manner, can regulate the immune function of cancer patients and inhibit tumor growth *via* binding to the opioid receptors.

Traditionally, research with MENK has focused on its analgesic activity. However, the observation of its immunoregulatory and anti-cancer activity, has suggested potential utility for the treatment of immune-related diseases and neoplasia. Since 2010, our research team has published a number of articles [19,33,41–42,65–68] elucidating the role of MENK in cancer biotherapy as an immunomodulatory drug (Fig. 1). Herein we summarize recent research from our laboratory and others on the bioactivity of MENK, with a focus on immunoregulation and cancer therapy to provide baseline information to further the study of MENK.

2. Immunoregulation by MENK

The endocrine system can regulate the immune system by controlling the expression of signaling molecules that act as activating agents, while providing feedback to the endocrine system. In addition, there are interactions within immune cells through cytokine network loops. This concept contributes to our understanding of the role MENK has in regulating the immune system. There are 3 types of opioid receptors: mu, delta, and kappa. The mu receptor is responsible for addiction and pain, while kappa and delta can regulate immunity. At certain concentrations,

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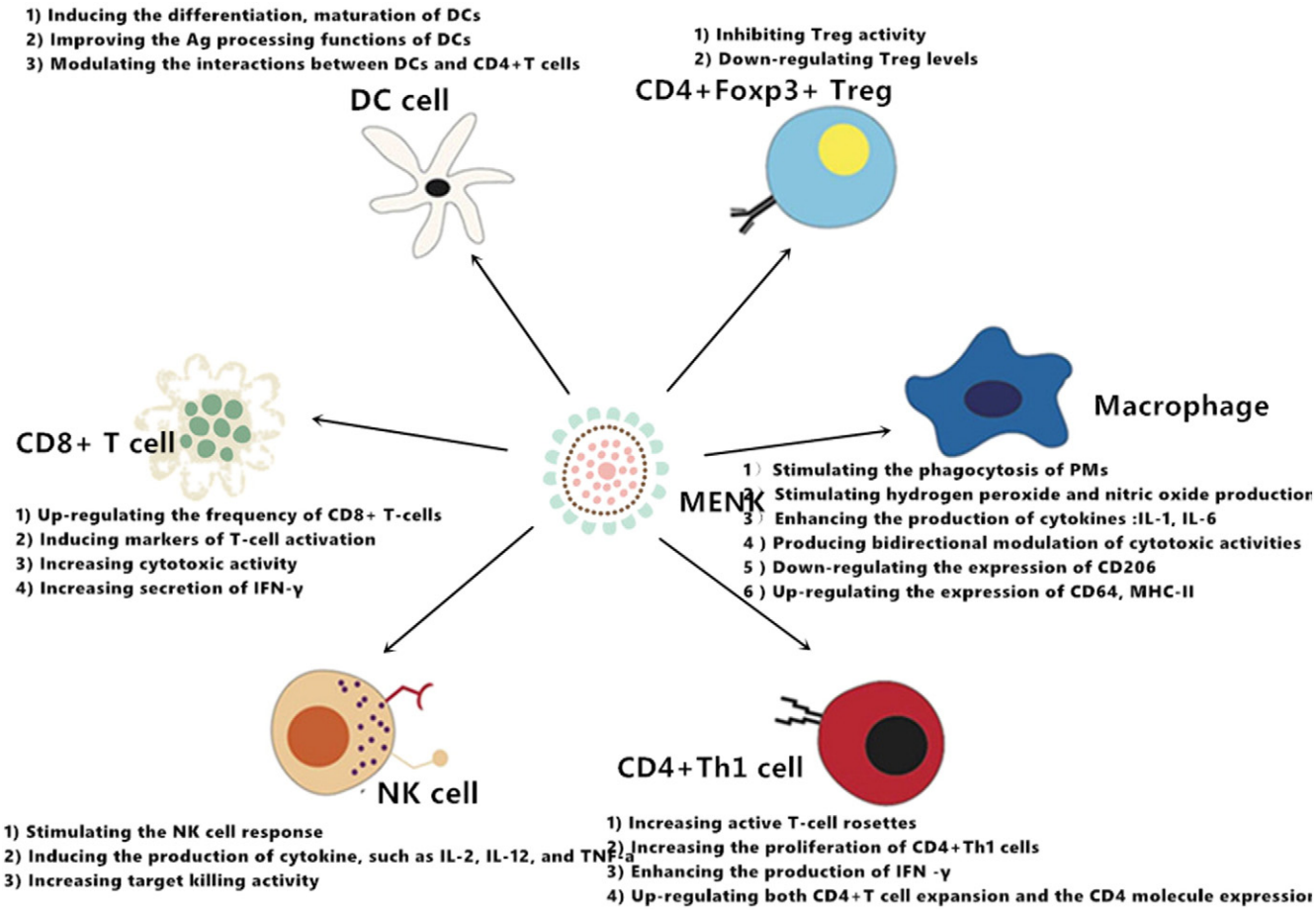

Fig. 1. Schematic diagram for immuno-regulation of MENK.

MENK binds to kappa or delta receptors on immune cells, rather than the mu receptor, resulting in immune augmentation [5].

2.1. Macrophages

Macrophages are specialized phagocytes that can scavenge microbes and cellular debris, and secrete immunomodulatory cytokines. They adapt their phenotypes to their microenvironment and are conventionally subset phenotypically into M1 (classically activated) or M2 (alternatively activated) macrophages. The M1 phenotype is considered to be pro-inflammatory, associated with T-helper (Th)-1 responses and the secretion of bactericidal factors in response to lipopolysaccharide and interferon γ (IFNγ) exposure. M2 macrophages exhibit a Th-2 cytokine expression pattern and are immunosuppressive [6].

In 1984, MENK induced alterations of macrophage functions were reported [7]. MENK was shown to exert a bimodal effect on rat peritoneal macrophages (PM). MENK at a low concentration (10^{-9}–10^{-7} M) was shown to increase antibody dependent cellular cytotoxicity (ADCC) with a simultaneous decrease in Fc gamma receptor (Fc gamma R) medicated phagocytosis, while the opposite response was observed at higher concentrations (10^{-6}–10^{-5} M) [8]. In the low concentrations, MENK appears to act on specific delta opioid receptors and its activity was positively coupled to guanylate cyclase levels. At relatively higher concentrations, MENK activity was not mediated by specific delta opioid receptors and appeared to occur *via* a Ca influx, adenylate cyclase activation as well as the processing of hormones by PM-enkephalinase [8]. Cell surface ligand-receptor interactions have a central role in the regulation and expression of macrophage function, and opioid receptors are included among these macrophage membrane receptors [9–10]. Recent studies concluded that MENK can stimulate the phagocytosis of PMs [11] and when combined with different opioid receptors, MENK has been shown to stimulate hydrogen peroxide and nitric oxide production by macrophages [12–14]. In addition, MENK has been shown to enhance the production of cytokines from macrophages, such as IL-1, IL-6, and to produce bidirectional modulation of cytotoxic activities by macrophage [15–18].

Our research indicates that MENK down-regulates the expression of CD206 and the production of arginase-1 (markers of alternatively activated (M2) macrophage) in tumor-associated macrophages *in vivo*, while it up-regulates the expression of CD64, MHC-II, and the production of induced nitric oxide synthase (markers of classically activated (M1) macrophages). Furthermore, studies on bone marrow-derived macrophages treated with MENK (10^{-12} M) *in vitro* demonstrated that MENK increases tumoricidal activity [19]. MENK can also enhance the secretion of reactive oxygen species and the production of interleukin-12 p40, tumor necrosis factor-α, while decreasing the production of interleukin-10 [19]. In conclusion, MENK effectively reduces M2 macrophages polarizing to a M1 macrophage phenotype, modulating a bias to a Th1 response, supporting MENK as a therapeutic agent for cancer.

2.2. T lymphocytes

In 1979, studies were reported that provided evidence for MENK receptors on normal human blood T lymphocytes, supporting a connection between the central nervous and immune systems [3]. Further research shows that MENK had pleiotropic effects on immune T-cell function. Molecular imaging demonstrated the regulated expression of both delta and kappa opioid receptors, predominantly on T-cells. MENK was shown to increase active T-cell rosettes on T-cells from lymphoma patients and normal volunteers [20–23]. Early studies conclude that MENK can both