Introduction

1.1 INTRODUCTION

Cancer is no longer considered by scientists just a jumble of mutated cells. It is defined as general name for more than 100 medical conditions involving uncontrolled and dangerous cell growth. Majority of death happens in cancer are not due to the primary tumor but rather to the situation called metastasis. Metastasis is the process by which a tumor cell leaves the primary tumor, travels to a distant site via the circulatory system and establishes a secondary tumor. Detection of cancer at an early stage before it has spread is possible to treat successfully by surgery/ local irradiation or by chemotherapy. However, in most cases when "primary" tumor is detected, cancer cells have already moved from their primary site and settled in other organs to continue their secondary growth gaining a "head-start" in the race against malignancy and in this situation patients rarely win (Chambers et al., 2002; Bagi, 2002).

Metastasis consists of five basic steps which are collectively termed as metastatic cascade (Mathias & Thomas, 2009; Chambers et al., 2002). Various steps which are involved in this process are 1) invasion and migration, 2) intravasation, 3) circulation, 4) extravasation and 5) colonization, proliferation and angiogenesis. The first step, invasion and migration involve detachment of individual cells from the primary tumor and invade adjacent, healthy tissue. These processes lead to secretion of several lytic enzymes which degrade the ECM (extracellular matrix) and, therefore, facilitate migration. A second step called intravasation allows intrusion of cancer cells into the blood and lymphatic vessels. After the attachment on the endothelial cells via adhesion molecules, the neoplastic cells secrete proteolytic enzymes which enable them to enter the blood vessel. Circulation is the third step in which the aberrant cell travels via the blood stream and has to withstand the conditions present in the blood. These are toxic for cancer cells due to the high concentration of oxygen and cytotoxic lymphocytes. A selection for particularly resistant and aggressive tumor cells takes place during this step. In fourth step of extravasation, the cells often get stuck in the capillaries of an organ and leave the blood stream by penetrating the endothelium through proliferation and/or proteolytic enzymes. Colonization, proliferation and angiogenesis is the final step in which the neoplastic cell settles at a distant tissue site and builds a secondary tumor. The later proliferates and induces neoangiogenesis in order to ensure sufficient vascularization.

In the early 1980s, it was postulated that blocking tumor invasion and metastases might be a promising therapeutic alternative to the classical anticancer drugs, which affected all types of proliferating cells, including normal cells (Sylvie et al., 1997). Thus, understanding the molecular mechanisms that determine tumor invasion and metastasis became a major challenge for cancer research. Tumor cell metastasis is a complex cascade of sequential steps, each of which is not yet fully understood. Progress has been made in identifying several key activators, one of which is the extracellular matrix (Engbring & Kleinmann, 2003). Invasion of the basement membrane (BM) by cancer cells was identified as a critical step in the metastatic process and therefore as an ideal target to block malignant progression (Sylvie et al., 1997). BM is a specialized extracellular matrix which separates tissue compartments from each other and acts as a physical barrier against passive cell diffusion. During the various stages of metastasis, metastasizing tumor cells encounter various host cells (platelet, lymphocytes, or endothelial cells) and/or extracellular matrix (ECM) components [fibronectin vitronectin and laminin] (Saiki et al., 1989). The adhesive interaction of tumor cells with components of ECM is regulated by various adhesion molecules and blocking of these interactions should prevent tumor metastasis (Woodhouse et al., 1997).

In the process of cancer cell invasion and dissemination, intra-cellular and cell–ECM interactions are of great significance. These functions are mediated by many cell adhesion molecules (type I and IV collagens, laminins, heparin sulfate proteoglycan, fibronectin, noncollagenous glycoproteins) and cell surface receptors (Miyasaka, 1995; DeRoock et al., 2001). Cell adhesion to the laminins, fibronectin and collagens is mediated in part by a group of heterodimeric transmembrane proteins called integrins, which are composed of a non covalently associated α and β subunit that define the integrin ligand specificity (DeRoock et al., 2001). Among the non-integrin receptors, the 67 laminin receptor (LR) is a 37/ 67 kDa laminin-binding protein that selectively binds a sequence of the laminin β1 chain, the YIGSR peptide. This receptor is overexpressed on the surface of a variety of tumor cells (Vanessa et al., 2007). The characteristic core sequence present in adhesion proteins (laminin, fibronectin, vitronectin) which demonstrated to contribute cell adhesion, spreading and migration of cells after interaction with active site present on receptors has been identified with use of proteolytic fragments, recombinant proteins, and synthetic

peptides (Engbring & Kleinmann, 2003). Synthesized peptide of similar core sequence showed increase in cell attachment, inhibited cell adhesion to extracellular matrix molecules by competitively disturbing the function of integrins on the cell surface as substrate analogs (Kenichiro et al., 1997).

The Tyr-Ile-Gly-Ser-Arg (YIGSR) and Glu-Ile-Leu-Asp-Val (EILDV) are pentapeptide sequence found in β1 chain of laminin and alternatively spliced segment of fibronectin respectively. Both YIGSR and EILDV is reported to be recognized by 67 kDa laminin receptor and integrin receptor (α4β1 & α4β7) respectively, which exhibited antimetastatic effect by virtue of their receptor binding ability resulting in to inhibition of cell migration and adhesion (Iwamoto et al., 1987, Hynes, 1992). However, a large amount of peptide is needed to obtain the inhibitory effect *in vivo* because of the poor stability due to its enzymatic degradation and rapid renal excretion from blood (Yu et al., 1999).

Recently, polymeric micelle has emerged as novel nanomedicine platform for drug delivery due to their size, stability, versatility and biocompatibility (Damon et al., 2007). One strategy to achieve cancer targeted drug delivery is the utilization of unique molecular markers that are specifically overexpressed within the cancerous tissues. Surface decoration of micelles with use of peptides like YIGSR-NH_2 or EILDV-NH_2 can represent a potentially feasible method for increasing the site specificity and efficacy of micelles containing anticancer drugs to tumor. The binding of YIGSR-NH_2/ EILDV-NH_2 conjugate micelles to the laminin and integrin receptor can cause receptor mediated internalization of carrier for intracellular drug delivery. Moreover, compared to monoclonal antibodies as ligand, peptides have some advantages like low immunogenicity and toxicity, a high stability and low cost (Lopez et al., 2004).

In present study, etoposide (ETO) was used as anticancer drug, which is a semi synthetic podophyllotoxin derived from the root of podophyllum peltatum effectively used for the treatment of lung cancer, testicular cancer, lymphoma and several type of leukemia. ETO is known to cause single-strand breaks in DNA and DNA damage through inhibition of topoisomerase II. It is cell cycle phase specific with predominant activity occurring in late S-phase and G2. An effort has been carried out to formulate

ETO loaded polymeric micelles of poly(ethylene glycol)-b-poly(caprolactone) micelles conjugated with peptide YIGSR-NH_2/ EILDV-NH_2 for effective treatment and prevention of metastasis.

1.2 AIMS AND OBJECTIVES

The purpose of this study was to design a novel ETO loaded block copolymeric micellar formulation with surface conjugation of peptide (YIGSR-NH_2/ EILDV-NH_2) in order to increase the tumor selectivity and efficacy.

- Synthesis and characterization of MPEG-PCL [Methoxy poly (ethylene glycol)-b-poly(ε-caprolactone)] and HOOC-PEG-PCL [Carboxyl poly(ethylene glycol)-b-poly(ε-caprolactone)] di-block copolymer of various blocks length.
- Formulation and optimization of ETO loaded MPEG-PCL micelles based on particle size and percent drug entrapment.
- Evaluation of MPEG-PCL micelles for critical micelles concentration, PEG surface density, fixed aqueous layer thickness, *in vitro* stability and hemolytic potential.
- Formulation and optimization of functionalized micelles conjugated with peptide (YIGSR-NH_2/ EILDV-NH_2) on the surface of micelles by post insertion technique.
- Characterization of micelles for DSC, X-RD, TEM
- To carry out lyophilization of formulation and *in vitro* drug release study at pH 7.4 phosphate buffer saline.
- Evaluation of stability study at various environmental conditions.
- *In vitro* cell line studies using highly metastatic B16F10 cell lines.
- *In vivo* studies i.e. experimental metastasis study and biodistribution study in mice models.

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