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Comparative assessment of lipid based nano-carrier systems for dendritic cell based targeting of tumor re-initiating cells in gynecological cancers

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ABSTRACT

We aimed to identify an optimum nano-carrier system to deliver tumor antigen to dendritic cells (DCs) for efficient targeting of tumor reinitiating cells (TRICs) in gynecological malignancies. Different lipid based nano-carrier systems i.e. liposomes, ethosomes and solid lipid nanoparticles (SLNPs) were examined for their ability to activate DCs in allogeneic settings. Out of these three, the most optimized formulation was subjected for cationic and mannosylated surface modification and pulsed with DCs for specific targeting of tumor cells. In both allogeneic and autologous trials, SLNPs showed a strong ability to activate DCs and orchestrate specific immune responses for targeting TRICs in gynecological malignancies. Our findings suggest that the mannosylated form of SLNPs is a suitable molecular vector for DC based therapeutics. DCs pulsed with mannosylated SLNPs may be utilized as adjuvant therapy for specific removal of TRICs to benefit patients from tumor recurrence.

1. Introduction

Gynecological cancers are a group of malignant disorders that occur in the organs of female reproductive tract including ovary, vulva, fallopian tube, and cervix. These cancers contribute around 25% of all the cancer incidences in Indian women. Amongst which, cancers of cervix, ovary and corpus uteri comprising 55.5%, 26.1% and 12.4% cases respectively, are reported to be most dominating and fatal (National Cancer Registry Programme, 2013; Takiar and Kumar, 2014). In-spite of numerous efforts one of the major reasons for this high mortality rate is the stage dependent therapeutic outcome of these cancers. From past epidemiological records, it is clearly understood that majority of female reproductive tract malignancies are broadly recorded in aged women (age: around 50). Of late, a significant surge in the rate of incidence of these malignancies in young women is a cause of serious concern in the sub-continent. The percentage of therapeutic cure is observed to be inversely related to the aggressive state of the disease, for example in case of cervical cancers, it drops to 15% in advanced stage from 45 to 80% for earlier stages. Unfortunately, majority of women suffering from these malignancies are diagnosed late at advanced malignant stages (Munoz et al., 2002; Vaughan et al., 2011; Fader et al., 2013).

As observed in the case of other cancers, the therapeutic options for these cancers are limited to radiation therapy, chemotherapy and surgical resection. In-spite of side effects to healthy cell population, late detection of gynecological cancers limits the efficiency of these therapeutic regimens and results in re-occurrence of the disease (Lengyel, 2010; Bellati et al., 2013). This re-occurrence is mainly attributed to the tumor re-initiating cells (TRICs), which sur-

Abbreviations: Ag, antigen; ANOVA, analysis of variance; CD, cluster of differentiation; C-SLNP, cationic solid lipid nanoparticles; DC, dendritic cells; ETSM, ethosomes; FITC, florescence isothiocyanate; GM-CSF, granulocyte macrophage colony stimulating factor; IFN, interferon; IL, interluekin; LDH, lactate dehydrogenase; LPSM, liposomes; ManDOG, monomannosyl-dioleyl glycerol conjugate; MHC, major histo-compatibility complex; M-SLNP, mannosylated solid lipid nanoparticles; NP, nanoparticles; PBS, phosphate buffer saline; PCR, polymerase chain reaction; PI, poly dispersity; SLNP, solid lipid nanoparticles; SPS, soya phosphatidyl choline; TAA, tumor associated Ag; TGF, tumor growth factor; TL, tumor lysates; TNF, tumor necrosis factor.

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vive conventional treatment, reside at certain imprecise places and facilitate re-growth of tumor form on cessation of the treatment (López et al., 2013). Studies indicated that TRICs exhibit stem cell characteristics and are competent enough to re-grow a tumor, comprising a heterogeneous group of daughter cells with self-renewal ability (Murphy, 2010). This re-growth is also accompanied by metastasis and therapeutic resistance which further worsens the disease outcome. In addition, the substantial toxicity induced by traditional regimens, add the TRICs escape from immunological surveillance (Ahmed et al., 2010; Davidson et al., 2015). Therefore, it is now essential to re-design the existing treatment options and focus on the alternate targeted strategies which can re-program immune system and results in better disease management to prevent cancer recurrence and extend survival. In this regard, utilizing patients own immune cells for therapeutics is an optimum strategy for decreasing the disease burden of reproductive tract cancers among females. It may also establish a sustained immune response against recurrence of cancer cells leading to long-term "cures" for patients of female reproductive cancers (Michelin and Murta, 2012).

Dendritic cells (DCs) being the professional antigen (Ag) presenting cells of the body represents an optimum source for the autologous therapeutic vaccine to selectively remove unwanted tumor cells (Bhargava et al., 2012). DCs possess a unique inherent ability to uptake Ag for processing and presenting them to T-cells for generation of Ag specific responses. The strategy has quickly caught interest of researchers and thus has been analyzed in different cancers (Bhargava et al., 2013a). Although proved clinically safe and effective, the success of these vaccines is clinically limited. Similar to the other cancer therapies, these vaccines too face certain cancer cell generated barriers which diminishes their capacity to eradicate tumor cells completely. The much complex tumor microenvironment organizes its surroundings in a way that favors tumor cell growth and suppresses immune effectors, thereby helping them to escape immunological surveillance (Bianchi et al., 2011; Latha et al., 2014). This escape is mainly supported by heterogeneous tumor cell population with highly mutated Ag, weak MHC expression, suppressive cells like Tregs, myeloid-derived suppressor cells and cytokines like TGF, IL-6 and IL-10 etc. which together generate an imbalance to improve tumor cell survival and limits the success of DC vaccines (Rolinski and Hus, 2014; Vasaturo et al., 2015). Therefore, it is now necessary to design and incorporate certain novel strategies with the existing DC vaccine designs. This can be accomplished by assimilating tumor Ag encapsulation through non-toxic and safe nano-materials. Nano-encapsulation will result in sustained and prolonged antigenic release. This will not only be helpful to improve effective Ag cross-presentation on DC surface but also rescue immune cells from tumor induced suppression (Zhang et al., 2012; Bhargava et al., 2013b). In addition to its basic applications, surface modification for direct DC targeting will also improve its wider therapeutic utility. A major question to be answered for developing such strategies is the selection of an optimum tumor associated Ag (TAA) and efficient nano-carrier based encapsulation system. In present work, we performed a comparative assessment of different lipid based nano-carrier systems to identify that whether nano-engineering DCs helps to selectively remove tumor cells of female reproductive tract. Moreover, tumor cell derived lysates were used as a source of Ag as they express many known and unknown tumor Ags (Lasky et al., 2013; Kandalaft et al., 2013). Earlier, we and others have reaffirmed that in comparison to sole Ags and other whole tumor cell based approaches, tumor cell lysates are more immunogeneic in nature (Bhargava et al., 2012; Ramanathan et al., 2014). The lipid based nano-carrier systems were selected due to their well documented clinical efficiency, superior physiochemical properties including biocompatibility, low diffusivity and ability to activate phagocytosis (Mishra et al., 2010a, 2012; Bhargava et al., 2013b, 2014).

2. Materials and methods

2.1. Work plan

The experiments were conducted in two parts. Firstly, a comparative assessment of nano-carriers was done to identify an optimum nano-carrier with maximum DC activation ability. For this different nano-carriers [Liposomes (LPSM), Ethosomes (ETSM) and Solid lipid nanoparticles (SLNPs)] were prepared using allogeneic cellular lysates obtained from female reproductive tract cancer cell lines and their ability to activate DCs for uptake and maturation was analyzed. While in second part, the identified nano-carrier with optimum DC activation ability was prepared using patients autologous tumor cell lysates, subsequently surface modified (cationic and mannosylated) and incubated with autologous DCs. Thereafter, the ability of DCs to stimulate specific immune responses for selectively tumor cells targeting was examined.

2.2. Cell lines and patient samples

For initial allogeneic experiments female reproductive tract cancer cell lines SK-OV-3: ovarian adenocarcinoma; KLE: endometrial adenocarcinoma; and HeLa: cervical adenocarcinoma was used to obtain cellular lysates. Later, autologous tumor lysates (TL) were collected from surgically removed tumors of 10 female reproductive tract cancer patients (3 ovary, 4 endometrial and 3 cervical). Autologous DCs were isolated by using 20 mL blood collected 1–2 h before surgery, while allogeneic DC isolation was done from blood obtained from healthy volunteers.

2.3. Generation of DCs

Allogeneic and autologous DCs were generated from a concentrated leukocyte fraction isolated from peripheral blood by following the protocol standardized earlier. In brief, peripheral blood was centrifuged against density gradient to separate mononuclear cells and the cells were incubated in RPMI media overnight to allow plastic adherence. Following incubation the adherent cells were washed and cultured with GM-CSF (500 U/mL) and IL-4 (8 ng/mL) enriched RPMI media at 37 °C for 7 days (Bhargava et al., 2013b).

2.4. Tumor cell lysates for encapsulation

Whole tumor cell lysates were generated using repeated freeze-thaw cycles and sonication method. Following which, the cells were centrifuged to remove debris and the protein content was analyzed photometrically. Silver staining was performed to characterize the obtained lysate. For downstream analysis, these lysates were then mixed with detector dye i.e. FITC at 1:1 ratio and incubated at 4 °C for 12 h. Dialysis was performed to remove any unbound dye (Bhargava et al., 2013b).

2.5. Preparation of different nano-carriers

2.5.1. Liposomes

Liposomal encapsulation of TLs was done following our previously published protocol. Briefly, an ethanolic solution of SPC was mixed with Span 80 (ratio 86%:14% wt/wt) in TL containing phosphate buffer (pH 6.5) (10 μg/mL). Following which the suspension was filtered by 0.22 μm polycarbonate filters (Millipore, Billerica, Massachusetts) (Mishra et al., 2010a).