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Dendritic cell–tumor cell hybrids and immunotherapy: what's next?

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Abstract
Dendritic cells (DC) are professional antigen-presenting cells currently being used as a cellular adjuvant in cancer immunotherapy strategies. Unfortunately, DC-based vaccines have not demonstrated spectacular clinical results. DC loading with tumor antigens and DC differentiation and activation still require optimization. An alternative technique for providing antigens to DC consists of the direct fusion of dendritic cells with tumor cells. These resulting hybrid cells may express both major histocompatibility complex (MHC) class I and II molecules associated with tumor antigens and the appropriate co-stimulatory molecules required for T-cell activation. Initially tested in animal models, this approach has now been evaluated in clinical trials, although with limited success. We summarize and discuss the results from the animal studies and first clinical trials. We also present a new approach to inducing hybrid formation by expression of viral fusogenic membrane glycoproteins.

Key Words: *cancer immunotherapy, cell fusion, dendritic cells, fusogenic membrane glycoproteins*

Introduction

Cancer therapy relies largely on surgery, radiation and/or the use of chemotherapeutic drugs. The emergence of resistant tumors is one of the challenges currently facing some cancer patients. This has prompted the evaluation of alternative approaches such as immunotherapy. This strategy aims at inducing an immune response leading to the destruction of tumor cells. Extensive studies based on experimental animal models have provided significant insights into the anti-tumor immune responses (1–3). The proliferation of tumor cells is associated with the death of a fraction of them, and therefore with the release of tumor antigens that can then be captured by immature dendritic cells (DC). Strategically localized in many tissues, DC play the role of sentinels of the immune system. Upon antigen capture, DC can mature and migrate to the lymph nodes, where they can present tumor antigens associated with major histocompatibility complex (MHC) molecules to T lymphocytes (4). T cells expressing a specific T-cell receptor (TCR) receive a second signal of co-stimulation, through the interaction of the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) expressed by activated DC, with CD28 expressed by T lymphocytes. The engagement of CD40 ligand on T cells with its receptor CD40 expressed by DC promotes the reciprocal activation of these two cell types. Activated T cells secrete cytokines, such as interleukin (IL)-2 and interferon-gamma (IFN-γ), allowing for their proliferation and differentiation in cytotoxic T cells (CTL) and leading to the activation of non-specific effectors of the immune response, such as macrophages (5) and natural killer cells (NK) (6). This activation is sustained by several other co-signals between T cells and DC, such as 4-1BB engagement [a tumor necrosis factor (TNF) family member] with its ligand on activated DC (7). Others receptors have been identified for their potential to promote anti-tumor immunity, including glucocorticoid-induced TNF receptor (GITR) and CD27, members of the TNF receptor superfamily, and inducible co-stimulatory molecule (ICOS), another member of the B7 family. The ligands for these receptors, GITR ligand (GITRL),

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CD27 ligand (CD70) and ICOS ligand (ICOSL; B7h), are predominantly expressed on antigen-presenting cells (APC) (B cells, macrophages and DC) (8,9). CD27/CD70 engagement can promote tumor rejection by promoting both NK-dependent and T cell-dependent anti-tumor mechanisms (10–12). ICOSL expression on tumor cells promotes regression in fibrosarcoma and plasmocytoma models via CD8-dependent mechanisms (13,14). GITR may also be endowed with anti-tumor effects as administration of anti-GITR agonist antibody (Ab) may curtail tumor growth in several murine tumor models (15,16).

Unfortunately, in many experimental animal models and in the vast majority of cancer patients, anti-tumor immunity is impaired by multiple mechanisms of tumor-induced immunosuppression/tolerance, resulting in tumor cell outgrowth and eventually death. Our team has demonstrated that the blockade of DC maturation in a non-functional stage may contribute to these phenomena of tumor escape from immune elimination. This inhibition of DC by cancer appears irreversible, even in the presence of various activators such as TNF-alpha (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF) and lipopolysaccharides (LPS) (17). Extensive studies focusing on the optimization of DC precursor isolation, DC differentiation and antigen loading have new protocols of immunotherapy using *ex vivo* DC with (a) necrotic or apoptotic tumor cells (18–20) or (b) tumor-associated antigen peptides (18,21–35), or (c) by electroporation of total tumor antigen RNA (36–42) or tumor-associated antigen (TAA) encoding mRNA (43–48). These loaded DC are then re-injected into cancer patients (49,50).

However, only limited clinical responses have been observed in cancer patients treated with DC-based vaccines. There is therefore a critical need to consider novel strategies for DC loading, differentiation and activation. One such alternative consists of the fusion of DC with tumor cells. These hybrid cells express the MHC class I and II molecules associated with tumor antigens and co-stimulatory molecules, and thus may be capable of activating both specific $CD4^+$ and $CD8^+$ T lymphocytes (Figure 1).

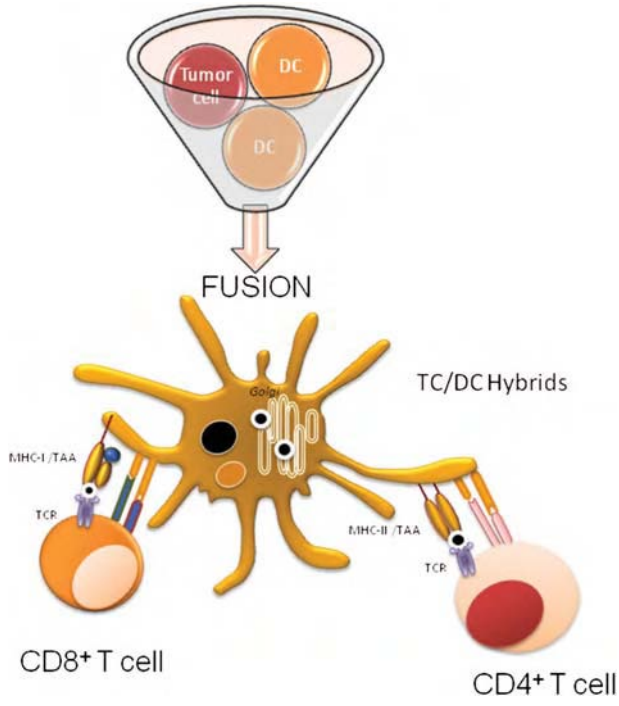

Figure 1. TAA presentation by DC–tumor cell hybrids. Fusion of cytoplasms leads to the sharing of DC and tumor cell proteins and the antigen presentation machinery. So, TAA can be processed and presented to T cells, in association with MHC class I and class II. Thus hybrids are powerful activators of the immune response against tumor cells.

Animal studies

The first use of cell–cell fusion to treat tumors involved BERH-2 hepatocellular carcinoma cells and activated B cells (51). In this study, Guo *et al.* (51) showed that polyethylene glycol (PEG)-induced hepatoma–B-cell hybrids were able to protect rats against parental hepatocarcinoma cells. All rats injected intrahepatically twice with 1×10^6 hybrid cells remained tumor free for 180 days, even after a rechallenge with parental BERH-2 cells. Immunization with hepatoma–B-cell hybrids also led to eradicating established hepatomas. Immunization with hybrids of rats injected or surgically implanted with hepatoma into the liver promoted overall survival (100% and 75%, respectively). It was demonstrated that hepatoma–B-cell hybrids developed into tumors in either anti-CD4 or anti-CD8 Ab-treated rats. In contrast, if CD4 or CD8 T-cell depletions were performed 14 days after immunization with hybrids, challenges with parental BERH-2 cells resulted in tumors only in the anti-CD8 Ab-treated group. Thus, hepatoma–B-cell hybrids may be capable of inducing and sustaining a T-cell dependent immune response against carcinoma.

The pioneer study report the effects of these tumor cell–DC hybrids was published by Gong *et al.* (52) in 1997. The authors demonstrated the superiority of DC–tumor cell hybrids to induce a specific anti-tumor immune response. *In vitro*, flow cytometry analyzes demonstrated that fused cells/mucin-1 (FC/MUC1) co-expressed MHC class I and II, B7-1 and -2 co-stimulatory molecules, Inter-Cellular Adhesion Molecule 1 (ICAM-1) and the MUC1 antigen previously stably transfected in MC38 cells. FC/MUC1 hybrids appeared to be non-tumorigenic and able to induce an immune response. *In vivo*, no tumor was formed in mice injected with FC/MUC1, indicating