Dendritic cell therapy for cancers and underlying mechanisms involved in cancer development

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Abstract:

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) that affect prime naive T cells and create proper immune responses. The uncontrolled growth of cancer cells often results from the cell's successful inhibition of cytotoxic T lymphocytes. In addition to conventional cancer treatments, including surgery, chemotherapy, and radiation therapy, immunotherapies have been seen to offer promising and innovative cancer treatments. Currently, DC therapy is one of the most popular immunotherapies because it uses tumour antigen-pulsed DC vaccines to fight against cancers. Patients with end-stage cancers treated with DC therapy may extend survival significantly for up to 10 to 15 more years. Researchers worldwide, including in Vietnam, have been focusing on determining the etiology of cancers with an aim to control cancers. One of the major causes of cancer is an increased expressions of proteins, including cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD-1) which both affect the recruitment of a large number of regulatory T cells and abolishes the presence of cytotoxic T lymphocytes migrating to the tumor sites, and resulting in an immune tolerance. In addition to this, the abnormal activation of the nuclear factor-κB (NF-κB) is also an important risk factor for cancer, because its activation leads to transcription of nuclear genes involved in regulating the cell physiological processes, such as maturation, differentiation, proliferation, migration, and invasion. This NF-κB signal is modulated by bonds among most receptors on immune cell surfaces and their specific ligands. Therefore, investigations of the precise molecular mechanism associated with the regulation of cancer development by DCs and other leukocytes, and the efficiency of cancer therapies have been major challenges for scientists worldwide.

Keywords: ***antigen presentation, cancer, cytokine, dendritic cell, dendritic cell therapy, T-cytotoxic.***

Classification number: ***3.5***

Introduction

In many cancer cases, immune cells, particularly those of antigen-presenting cells, that have not performed their main functions, therefore tumours associated with antigens may not be presented to T and B lymphocytes successfully. Moreover, the expressions of ligand-blocking genes on cancer cell surfaces cause the inhibition of immune response, leading to an uncontrolled proliferation of cancer cells. Currently, several research studies have focused on immunotherapies using various immune cell types such as natural killer (NK) cells, dendritic cells (DCs), macrophages, and others to suppress the development and metastasis of cancers. However, DC therapy is one of the most common techniques used in cancer therapies because these cells display more predominant features than other immune cells do. DCs are the most specific tumour antigen-presenting cells to prime naive T lymphocytes initiating needed for immune responses. Mature DCs are characterised by secreting a larger number of inflammatory cytokines and chemokines to promote differentiations of T cells into effector cells. The DC-based cancer immunotherapy aims to induce a recurrence of immune responses in these patients. Accordingly, autologous DCs are pulsed with specific tumour antigens to be mature DCs, which are returned to the donors that show promising preliminary results to cancer treatments. In addition to the roles of DCs in inducing specific T-cytotoxic cells capable of seeking and destroying cancer cells including cancer stem cells, DCs exhibit their ability to activate immune responses as vaccines, resulting in preventing the risk of recurrence and metastasis of cancer cells. Besides this, further investigation of molecular mechanisms involved in regulation of the development of cancers is needed to determine the precise molecular etiologies causing cancers. Currently, issues have been considered extensively by scientists to determine particular answers as soon as possible.

DC biology

DCs are the most effective professional antigen-presenting cells to T lymphocytes to initiate the immune response and remain with immunological memory [1]. DCs are

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present in all lymphoid organs, including spleens, lymph nodes, subcutaneous tissue, intestines, bronchi, and lungs, and therefore these cells show their ability to capture exogenous antigens. In these peripheral tissues, DCs ingest these antigens by endocytosis to become mature DCs, which are characterized by (a) The up-regulation of cell surface molecules including major histocompatibility complex (MHC) and co-stimulatory markers CD80, CD86, CD40 and CD54; (b) The enhanced releases of inflammatory cytokine and chemokine productions of interleukin (IL)-12, IL-6, tumor necrosis factors (TNF)-α, and C-C chemokine receptor type 7 (CCR-7). Mature DCs lose their adhesion and are subsequently recruited to the secondary lymphoid organs to present the antigens to T lymphocytes and differentiate them into effector cells in immune response [1].

Besides this, DCs are also involved in maintaining immune tolerance when they consider antigens as endogenous factors. Similar to the ingestion of exogenous antigens, DCs capture the endogenous factors through their phagocytosis and then lose their adhesion. In contrast to the immunostimulatory effects of DCs, the induction of immune tolerance by DCs (i.e. tolerogenic DCs) is characterized by inhibiting an expression of cell surface molecules and an increase in anti-inflammatory cytokine productions such as IL-10 and transforming growth factor beta (TGF-β) to promote the differentiation of regulatory T cells (T reg) [1, 2]. By this way, the tendency of cancer cells is to invade the discovery of immune cells and subsequently proliferate to increase rapidly in number.

There are many different DC subtypes, including myeloid DCs (mDCs), that are derived from myeloid progenitor cells localised within the bone marrow microenvironment, plasmacytoid DCs (pDCs) are derived from lymphoid progenitor cells in lymphoid organs, and inflammatory DCs are derived from monocytes. The pDCs are only present in lymphoid organs and rarely expressed in other organs. They are recruited to the lymphoid organs, where they are in an inflammatory state [3]. The functional roles of the pDCs considered as both antigen-presenting cells and stimulators of differentiations of T lymphocytes into effector cells have been in debate and undefined. These DC subtypes share common features including adhesion, antigen-presentation, phagocytosis and migration, however, their immune responses, when exposed to various antigens, are subtype-specific properties [1]. The pDCs treated with viral antigens produce a large amount of interferon (IFN) type 1, such as IFN-α and IFN-β (therefore also known as IFN producing cells), and the mDCs exposed to microbial antigens secrete many inflammatory cytokines such as TNF-α and increase the nitric oxide (NO) synthesis. Various effects of cytokines and chemokines in promoting the differentiation of T lymphocytes into effector cells in the activation of immune response are different from each other [1].

DCs in cancer treatments

Cancer antigens are considered as endogenous factors, therefore, leading to the induction of immune tolerance. Cancer cells are characterized by rapid proliferation and spread throughout the body in the following tendencies: (a) Many T reg cells located at tumor sites contribute to their inhibitory effects on the proliferation of cytotoxic T cells, resulting in an invasion of cancer cells from the monitor of immune system induced by foreign antigens; (b) The up-regulation of inhibitory genes in cancer cells leads to suppressing the activation of signaling receptors on antigen-presenting cell surfaces, thus, these antigens are not presented to T lymphocytes; (c) The induction of cancer cells on the enhanced synthesis of anti-inflammatory cytokines such as IL-10 and TGF-β to suppress the differentiations of T lymphocytes and enhance the release of Fas Ligand (FasL) proteins, which triggers the activation of Fas/FasL signaling pathway in leukocytes causing the apoptotic cell death and inhibiting the immunity [4] (Fig. 1).

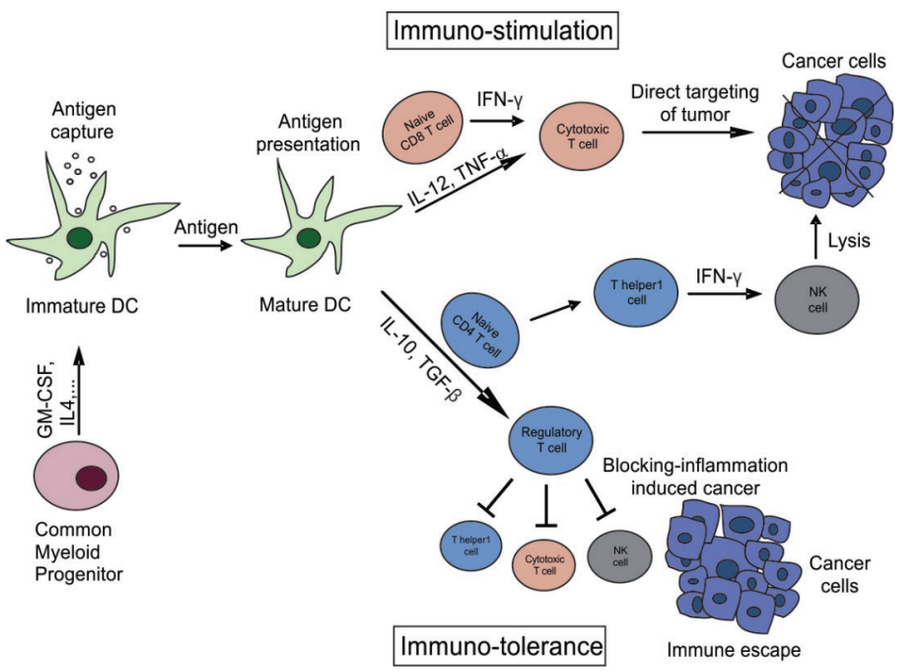

Fig. 1. The regulation of DCs in immune response against cancers.

From investigations using mature DCs as a vaccine, immature DCs should be stimulated with particular antigens derived from cancer cells and subsequently transfused back to their donors with an aim to eliminate cancer cells and prevent the risks of metastasis/ recurrence of malignant cells circulated in the body [1]. DC therapies against cancers have been used in clinical laboratory testing in many medical centers. These observations have focused on the identification of the most specific antigens derived from cancer cells to induce the activation of immune response by mature DCs, and followed by the identification of mechanisms underlying the pathogenesis of cancers and determination of targeted cancer therapies.

Technically, DCs are expanded ex-vivo for cancer treatments by using peripheral blood mononuclear cells (PBMCs) isolated from blood samples and then PBMCs, which are cultured in the presence of cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, TNF-α, and FMS-like tyrosine kinase 3 ligand (Flt3L), to attain DCs. The tumour peptide-induced mature DCs are returned to the donors with the aim to reduce the size of tumours and induce the immunity in patients with cancers. This is currently one of the most common technologies in cancer treatment.

Global researches on cancer

Since 1967, Prof. Okamoto, et al. (Japan), used OK-432 antigen derived from streptococcus to induce activation of DCs in eliminating cancers [5]. In 1995, the DC therapy using melanoma-associated antigen-derived epitopes were treated for patients with melanoma [6]. Currently, clinical applications of DC therapy in treatments of some cancers, such as pancreatic [7], bile duct [8], lung [9], and ovarian [10] cancers, has attained particular success. The results of which indicated that patients with late-stage cancers or metastatic cancers are treated with either chemotherapy or radiation treatment, and followed by DC therapy, could prolong the longevity as much as 10 to 15 years. In addition to this, other studies also revealed that patients treated with DCs with high MHC class I molecule expression showed a larger number of intraepithelial CD8+ T cells, resulting in improving survival even more [11, 12].

In addition to stimulation of DCs with tumour antigens, necrotic cancer cells have been used to stimulate DCs, which are transfused into tumor-bearing mice, attaining promising results to date [13]. These cells are exposed to ultraviolet light to kill targeted cancer cells and damage their DNA structures, leading to cell necrosis or apoptosis. The flow cytometry method uses annexin V antibody to detect the exposure of phosphatydilserine on the cell surfaces and 7AAD to stain necrotic cell nuclei with the aim to determine whether they are apoptotic or necrotic cells. Several reports indicated that cellular DNA damage of melanoma and B-cell leukemia to induce apoptotic cell death and be followed by exposure to DCs, which are then transfused into cancer patients, leads to activation of cytotoxic T lymphocytes-mediated immune response [14, 15]. In 1998, R.C. Fields, et al., demonstrated that mature DCs triggered by necrotic cancer cells, including breast cancer or sarcoma cells transfused into tumor-bearing mice, resulted in successful immune response to the second exposure of various cancers and inhibition of the metastasis of lung cancer [16]. Currently, the transfusion of mature DCs triggered by nontoxic-targeted cancer cells such as melanoma [17], breast [18], lymph node [19], and several cancers into tumor-bearing mice, has been achieving good consequences. However, no study indicates contributions of these mature DCs to patients with cancers by transfusing them directly to the donors.

Cancer research in Vietnam

In Vietnam, researchers of the Laboratory of Stem Cell Research and Application at the Vietnam National University, Ho Chi Minh City was the first to perform experiments using DCs in mice in 2010. Peripheral blood mononuclear cells (PBMCs) are isolated from blood samples and cultured in a specific pathogen-free condition to obtain therapeutic DCs. The DCs are stimulated with tumour antigens and the activated DCs are then transfused into breast cancer-bearing mice. As a result, the DC therapy, without using chemotherapy or radiation treatment, reduces 80% of the size of breast cancer [13, 20]. However, the application of DC therapy has not clinically been applied yet to treatments of breast cancer patients. In the future, further studies are needed to determine the etiology and molecular mechanisms involved in the regulation of pathogenesis of cancers and would be followed by the discovery of effective cancer drugs.

Molecular mechanisms involving in cancer development

The induction of up-regulated expressions of both cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD-1) proteins on the leukocyte surfaces by cancer cells is one of the most successful processes to multiply in number and immune escape. Therefore, a large number of T-regs and T helper 17 cells (Th17) are recruited to tumour sites, resulting in the development of immune tolerance and inhibition of the appearance of cytotoxic CD8 T cells and nature killer (NK) cells [21], which aim to suppress the immune response against cancers.

Besides, other studies showed that the expression levels of several receptors, such as toll-like receptors (TLR), nucleotide oligomerization domain (NOD)-like receptors (NLRs), and

TGF-β receptors (TGF-βR), all located at leukocyte surfaces are associated with the development of cancers [22]. Investigations of patients with prostate cancer revealed that the genetic alteration of some genes such as TLR4, TLR1, TLR6, and TLR10, are risk factors for cancers [23, 24]. Similarly, a nucleotide change in the TLR2 gene is also a risk factor for colorectal cancer in mice [25]. Studies on downstream molecules of TLR signalling including myeloid differentiation primary response protein (MyD88) demonstrated that inhibition of the expression of this gene reduces the development of several cancers such as colorectal cancer, melanoma, and liver cancer [26, 27]. The immunotherapy for acute myeloid leukemia using TLR signaling-mediated mature DCs induced by cancer antigens has been achieving high efficiency for the treatment of cancers [28]. Several investigations focused on the role of NLR signaling in the modulation of cancers, as alteration in Nod2 gene is at risk of colorectal cancer [29] and NLR family, pyrin-containing 3 gene (NLRP3)-deficient mice are susceptible to colon and colorectal cancers, mediated through the release of IFN-γ cytokine and activation of signal transducer and activator of transcription (STAT)-1 signaling [30]. In addition to this, a binding between TGF-β, a cytokine produced by leukocytes with TGF-βR, is used to stimulate activation of this signaling leading to blocking the development of early stage cancers; however, facilitating indirectly to the development of late-stage cancers by recruiting a large number of T regs to tumor sites [31]. The induced activation of downstream molecules of TGF-βR including Smad, inhibits the proliferation of cancer cells. Therefore, the abnormal expression of the Smad protein is also a risk factor for several cancers, such as colorectal, prostate, and head and neck cancers [32].

The bindings between receptors located on the leukocyte surfaces and specific ligands trigger activation of downstream molecules, such as mitogen-activated protein kinases (MAPK) and nuclear factor-κB (NF-κB) signalling pathways, is leading to the transcriptions of genes involved in the regulation of cellular physiological processes [33, 34]. Hence, the abnormal activation of NF-κB signalling causes about 20% of human cancers derived from patients with severe chronic diseases and activation of MAPK signalling in leukocytes, especially DCs, increases the immune response against cancers.

Conclusions

At present, immunotherapies for cancers have attracted special attention by global scientists due to their high safety, efficiency, and not causing suffering from side effects to the patients induced by conventional cancer therapies. The DC therapy technique considered as a vaccine in the treatment of cancers, has been applied extensively since DCs display predominant characteristics than those of other immune cells as follows, the most efficient processing and presenting capacities to T lymphocytes and the release of larger number of inflammatory cytokines compared to other antigen-presenting cells such as macrophages, B cells or NK cells. Therefore, the induction of differentiation of T lymphocytes into effector cells by activated DCs could result in most favourable immune responses. Combined DC therapy in the treatments of cancers after surgery, radiation and chemotherapy has been widely tested with aims for application across broad DC applications in preventing the development of malignant tumours, as well as inducing immunity against cancers. Similarly, the DC-therapy might suppress the threat of cancer recurrences and metastasis of malignant cancer cells to improve longer survivals in patients with end-stage cancers. Further investigations are needed to fine-tune DC protocols and figure out the most effective ways to abolish cancers and the DC therapy could be among the most promise immunotherapies in future treatments of cancers.

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REFERENCES

[1] J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, K. Palucka (2000), "Immunobiology of dendritic cells", *Annu. Rev. Immunol.*, **18**, pp.767-811.

[2] I. Volovitz, S. Melzer, S. Amar, J. Bocsi, M. Bloch, S. Efroni, Z. Ram, A. Tarnok (2016), "Dendritic Cells in the Context of Human Tumors: Biology and Experimental Tools", *Int. Rev. Immunol.*, **35**, pp.116-135.

[3] H. Yoneyama, K. Matsuno, Y. Zhang, T. Nishiwaki, M. Kitabatake, S. Ueha, S. Narumi, S. Morikawa, T. Ezaki, B. Lu, C. Gerard, S. Ishikawa, K. Matsushima (2004), "Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules", *Int. Immunol.*, **16**, pp.915-928.

[4] X. Yu, Y. Li, Y. Yu, J. Lei, G. Wan, F. Cao (2016), "Associations between FAS rs2234767 and FASL rs763110 polymorphisms and the risk of lung cancer: a meta-analysis of 39,736 subjects", *Onco Targets Ther.*, **9**, pp.2049-2056.

[5] H. Okamoto, S. Shoin, S. Koshimura, R. Shimizu (1967), "Studies on the anticancer and streptolysin S-forming abilities of hemolytic streptococci", *Jpn J. Microbiol.*, **11**, pp.323-326.

[6] A.B. Bakker, G. Marland, A.J. de Boer, R.J. Huijbens, E.H. Danen, G.J. Adema, C.G. Figdor (1995), "Generation of antimelanoma cytotoxic T lymphocytes from healthy donors after presentation of melanoma-associated antigen-derived epitopes by dendritic cells in vitro", *Cancer Res.*, **55**, pp.5330-5334.

[7] M. Okamoto, M. Kobayashi, Y. Yonemitsu, S. Koido, S. Homma (2016), "Dendritic cell-based vaccine for pancreatic cancer in Japan", *World J. Gastrointest. Pharmacol. Ther.*, **7**, pp.133-138.

[8] M. Kobayashi, T. Sakabe, H. Abe, M. Tanii, H. Takahashi, A. Chiba, E. Yanagida, Y. Shibamoto, M. Ogasawara, S. Tsujitani, S. Koido, K. Nagai, S. Shimodaira, M. Okamoto, Y. Yonemitsu, N. Suzuki, M. Nagaya, Therapy DC-vaccine study group at the Japan Society of Innovative Cell (2013), "Dendritic cell-based immunotherapy targeting synthesised peptides for advanced biliary tract cancer", *World J. Gastrointest. Surg.*, **17**, pp.1609-1617.

[9] H. Takahashi, M. Okamoto, S. Shimodaira, S. Tsujitani, M. Nagaya, T. Ishidao, J. Kishimoto, Y. Yonemitsu, Therapy DC-vaccine study group at the Japan Society of Innovative Cell (2013), "Impact of dendritic cell vaccines pulsed with Wilms' tumour-1 peptide antigen on the survival of patients with advanced non-small cell lung cancers", *Eur. J. Cancer*, **49**, pp.852-859.

[10] M. Kobayashi, A. Chiba, H. Izawa, E. Yanagida, M. Okamoto, S. Shimodaira, Y. Yonemitsu, Y. Shibamoto, N. Suzuki, M. Nagaya, Therapy DC-vaccine study group at the Japan Society of Innovative Cell (2014), "The feasibility and clinical effects of dendritic cell-based immunotherapy targeting synthesised peptides for recurrent ovarian cancer", *J. Ovarian Res.*, **7**, p.48.

[11] E. Sato, S.H. Olson, J. Ahn, B. Bundy, H. Nishikawa, F. Qian, A.A. Jungbluth, D. Frosina, S. Gnjatic, C. Ambrosone, J. Kepner, T. Odunsi, G. Ritter, S. Lele, Y.T. Chen, H. Ohtani, L.J. Old, K. Odunsi (2005), "Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer", *Proc. Natl. Acad. Sci. USA*, **102**, pp.18538-18543.

[12] J. Tel, G. Schreibelt, S.P. Sittig, T.S. Mathan, S.I. Buschow, L.J. Cruz, A.J. Lambeck, C.G. Figdor, I.J. de Vries (2013), "Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets", *Blood*, **121**, pp.459-467.

[13] S.T. Nguyen, H.L. Nguyen, V.Q. Pham, et al. (2015), "Targeting specificity of dendritic cells on breast cancer stem cells: in vitro and in vivo evaluations", *Onco Targets Ther.*, pp.323-334.

[14] O. Manches, G. Lui, J.P. Molens, J.J. Sotto, L. Chaperot, J. Plumas (2008), "Whole lymphoma B cells allow efficient cross-presentation of antigens by dendritic cells", *Cytotherapy*, **10**, pp.642-649.

[15] G. Pietra, R. Mortarini, G. Parmiani, A. Anichini (2001), "Phases of apoptosis of melanoma cells, but not of normal melanocytes, differently affect maturation of myeloid dendritic cells", *Cancer Res.*, **61**, pp.8218-8226.

[16] R.C. Fields, K. Shimizu, J.J. Mule (1998), "Murine dendritic cells pulsed with whole tumour lysates mediate potent antitumor immune responses in vitro and in vivo", *Proc. Natl. Acad. Sci. USA*, **95**, pp.9482-9487.

[17] N. Werthmoller, B. Frey, R. Wunderlich, R. Fietkau, U.S. Gaipl (2015), "Modulation of radiochemoimmunotherapy-induced B16 melanoma cell death by the pan-caspase inhibitor zVAD-fmk induces anti-tumor immunity in an HMGB1-, nucleotide- and T-cell-dependent manner", *Cell Death Dis.*, **6**, p.e1761.

[18] I.M. Meraz, D.J. Savage, V. Segura-Ibarra, J. Li, J. Rhudy, J. Gu, R.E. Serda (2014), "Adjuvant cationic liposomes presenting MPL and IL-12 induce cell death, suppress tumor growth, and alter the cellular phenotype of tumors in a murine model of breast cancer", *Mol. Pharm.*, **11**, pp.3484-3491.

[19] S.K. Hira, I. Mondal, P.P. Manna (2015), "Combined immunotherapy with whole tumour lysate-pulsed interleukin-15-activated dendritic cells and cucurbitacin I promotes strong CD8(+) T-cell responses and cures highly aggressive lymphoma", *Cytotherapy*, **17**, pp.647-664.

[20] P.V. Pham, N.T. Nguyen, H.M. Nguyen, L.T. Khuat, P.M Le, V.Q. Pham, S.T. Nguyen, N.K. Phan (2014), "A simple in vitro method for evaluating dendritic cell-based vaccinations", *Onco Targets Ther.*, **7**, pp.1455-1464.

[21] T. Kitamura, B.Z. Qian, J.W. Pollard (2015), "Immune cell promotion of metastasis", *Nat. Rev. Immunol.*, **15**, pp.73-86.

[22] H. Clevers (2004), "At the crossroads of inflammation and cancer", *Cell*, **118**, pp.671-674.

[23] E.M. El-Omar, M.T. Ng, G.L. Hold (2008), "Polymorphisms in Toll-like receptor genes and risk of cancer", *Oncogene*, **27**, pp.244-252.

[24] J. Sun, F. Wiklund, S.L. Zheng, B. Chang, K. Balter, L. Li, J.E. Johansson, G. Li, H.O. Adami, W. Liu, A. Tolin, A.R. Turner, D.A. Meyers, W.B. Isaacs, J. Xu, H. Gronberg (2005), "Sequence variants in Toll-like receptor gene cluster (TLR6-TLR1-TLR10) and prostate cancer risk", *J. Natl. Cancer Inst.*, **97**, pp.525-532.

[25] H. Yang, H. Zhou, P. Feng, X. Zhou, H. Wen, X. Xie, H. Shen, X. Zhu (2010), "Reduced expression of Toll-like receptor 4 inhibits human breast cancer cells proliferation and inflammatory cytokines secretion", *J. Exp. Clin. Cancer Res.*, **29**, p.92.

[26] W.E. Naugler, T. Sakurai, S. Kim, S. Maeda, K. Kim, A.M. Elsharkawy, M. Karin (2007), "Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production", *Science*, **317**, pp.121-124.

[27] S. Rakoff-Nahoum, R. Medzhitov (2007), "Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88", *Science*, **317**, pp.124-127.

[28] M. Subklewe, C. Geiger, F.S. Lichtenegger, M. Javorovic, G. Kvalheim, D.J. Schendel, I. Bigalke (2014), "New generation dendritic cell vaccine for immunotherapy of acute myeloid leukemia", *Cancer Immunol. Immunother.*, **63**, pp.1093-1103.

[29] J.H. Cho (2008), "The genetics and immunopathogenesis of inflammatory bowel disease", *Nat. Rev. Immunol.*, **8**, pp.458-466.

[30] M.H. Zaki, K.L. Boyd, P. Vogel, M.B. Kastan, M. Lamkanfi, T.D. Kanneganti (2010), "The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis", *Immunity*, **32**, pp.379-391.

[31] M. Saitoh (2015), "Epithelial-mesenchymal transition is regulated at post-transcriptional levels by transforming growth factor-beta signalling during tumour progression", *Cancer Sci.*, **106**, pp.481-488.

[32] J. Fang, H. Xu, C. Yang, S. Kayarthodi, R. Matthews, V.N. Rao, E.S. Reddy (2014), "Molecular Mechanism of Activation of Transforming Growth Factor Beta/Smads Signaling Pathway in Ets Related Gene-Positive Prostate Cancers", *J. Pharm. Sci. Pharmacol.*, **1**, pp.82-85.

[33] L.M. Coussens, Z. Werb (2002), "Inflammation and cancer", *Nature*, **420**, pp.860-867.

[34] F. Arce, G. Kochan, K. Breckpot, et al. (2012), "Selective activation of intracellular signalling pathways in dendritic cells for cancer immunotherapy", *Anti-cancer Agents Med. Chem.*, **1**, pp.29-39.