Radiation Therapy Effects on the Immune System

KATALIN LUMNICZKY AND GÉZA SÁFRÁNY
National Public Health Center, Department of Radiobiology and Radiohygiene, Budapest, Hungary

Abstract

Recent immunology research led to a drastic increase in the knowledge of antitumour immune response mechanisms and in parallel to a rapid development in various antitumour immune therapy strategies. This might hopefully result in the implementation of immunotherapeutic protocols within the standard anticancer regimens in the very near future. The similarly dynamic progress in the radiobiological knowledge proved that ionizing radiation does not have a general immune suppressing effect, as it has been thought for decades, but might possess certain immune stimulatory effects, as well. It is also known that local irradiation due to its out-of-field effects has systemic immune modulatory capacity, too. In the light of all these novel findings the optimal combination between antitumour immunotherapy and radiotherapy has become an increasing option. The present review summarizes the main antitumour immunological mechanisms that can be influenced by ionizing radiation.

Key Words: malignant tumours, radiation therapy, antitumour immunity

Corresponding author: Katalin Lumniczky,
National Public Health Center,
Department of Radiobiology and Radiohygiene,
Anna u. 5, Budapest, Hungary, H-1221
Phone: +36-1-482-2010
E-mail: lumniczky.katalin@osski.hu

Received: 10th October 2018
Accepted: 28th November 2018

INTRODUCTION

Local irradiation of the tumours is one of the most efficient antitumour treatment modalities. Radiation-induced tumour cell killing is the primary contributor to the success of radiotherapies. Radiation sensitivity of the tumour cells is influenced by many different factors such as the intrinsic radiation sensitivity of the tumour cells, the presence and extent of hypoxia within tumours and the proliferation capacity of the cells. The tumour microenvironment can also have serious effects on the radiation response of the tumours. Immune cells are important contributors of the tumour micro-environment; they can determine local inflammatory reactions and the type and extent of the antitumour immune response.

Radiation-induced bystander effects might also modify radiation responses both on the local and systemic levels. Bystander effects mean radiation-induced reactions in cells which were not hit directly by radiation. Although the cellular bystander signals are not well characterized so far, it is obvious that some of them (cytokines, chemokines, growth factors, etc.) mediate immune effects (Prise and O'Sullivan, 2009; Lumniczky and Sáfrány, 2015).

Antitumour immune therapy is perhaps the most dynamically evolving field of tumour treatments. It has dual aims: improving antitumour immune responses and inhibiting those pathways which are responsible for the immune suppressive effect of certain tumours. The new therapeutic modalities for instance immune therapy should always be combined with existing regimes such as radiation- and chemotherapies. Therefore we should understand how normal and malignant cells are responding to these combinations.

Formerly, it seemed obvious that high dose irradiations present immune suppressing effects (Cosimi et al., 1973). Recent findings however suggest that there is a dynamic relationship between radiation- and immune system effects: radiation might enhance certain immune responses while other immune pathways are suppressed.

The aim of the current review is to summarize our recent knowledge on radiation-induced immune effects.

RESULTS

Radiation effects on tumour cells

During oncogenesis different mutations will accumulate in tumour cells and that leads to the production of altered polypeptides. These polypeptides might bind on the cell surface to major histocompatibility receptor proteins which can lead to the activation of an antitumour immunity. During tumour progression, however the tumour cells can establish several mechanisms – increased proliferation capacity, decreased number of MHC molecules, shadowing of cell surface antigens, and production of immune suppressive cytokines - to evade immune recognition and that results in increasing immune suppression.

The effect of ionizing radiation on antitumour immunity strongly depends on radiation effects on the above mentioned mechanisms. Radiation usually induces mitotic cell death meaning that lethally damaged tumour cells might undergo several cell divisions before the eventual death. The most frequent forms of late mitotic death are necrosis and senescence. Slowly dying cells are still active metabolically and can produce several molecules capable to alter immune system reactions. These molecules (cytokines, chemokines, and small molecular weight stress molecules) can exhibit their action either locally or after entering the blood stream they might induce systemic immune effects. Occasionally, irradiated tumour cells might undergo a rapid cell death, mainly by apoptosis.

In the context of immunology we can distinguish either immunogenic or tolerogenic cell death mechanisms. During immunogenic cell death, molecules capable of activating the anti-tumour immune response are placed on the tumour cell surface and/or in the extracellular space. These molecules usually serve as danger signals which can increase the preparedness of the immune system mainly through the activation of dendritic cells (DC). Such danger signals are for instance the various heat shock proteins, such as Hsp70 and 90. The appearance of these proteins on tumour cell surfaces can improve the maturation of DCs and their capacity to recognize tumour antigens. They can also help the activation of CD8+ T and natural killer (NK) cells (Ma et al., 2010a). The first and perhaps the most important sign of the immunogenic cell death is the moving of the calreticulin molecule from the inner to the outer surface of the cells. It also causes immunogenic cell death if cellular ATP or DNA-bound HMGB1 (high mobility group protein B1) enters the extracellular space. HMGB1 can directly stimulate DC cells through TLR4 receptors. Free extracellular ATP also acts through DCs (Ma et al., 2011). During tolerogenic cell death dying tumour cells produce molecules which inhibit immune system activation. This process can be characterized by the increased level of CD47 protein on the surface of tumour cells. CD47, a molecule showing similarity to immunoglobulins, can bind to the SIRPα (signal regulatory protein alpha) receptor on the surface of macrophages inhibiting their phagocytic activity (Ma et al., 2010b). Increased CD47 level was detected in many types of tumours such as ovary, breast, colon, bladder and prostate tumours, hepatocellular carcinomas, gliomas as well as myeloid leukaemias (Jaiswal et al., 2009; Willingham et al. 2012). A tolerogenic effect was also reported for the CD39 molecule, which is usually present on the surface of regulatory T cells, but can appear on the surface of certain tumour cells (pancreatic tumours, melanomas), as well (Dzhandzhugazyan et al., 1998; Künzli et al., 2007). CD39 has the capacity to inhibit stress activated responses by degrading free ATP.

Radiation can influence both immunogenic and tolerogenic cell deaths. Radiation-induced increased calreticulin levels were detected in murine colon carcinomas, in fibrosarcomas and various melanoma derived cell lines (Obeid et al., 2007; Perez et al., 2009). In human papilloma virus-positive head- and neck tumours radiation therapy resulted in decreased CD47 cell surface expression (Vermeer et al., 2013). Soto-Pantoja et al. (2014) reported that blocking of CD47 on tumour cells and their microenvironment increased their radiation sensitivity and helped tumour invasion by cytotoxic CD8+ T cells.

As mentioned before radiation might induce necrotic cell death and senescence in both normal and tumour cells. Necrotic death of malignant cells is usually an immunogenic death if

induced by stress factors. In this case many danger signals and inflammatory cytokines are produced rapidly which can activate the immune system through DCs (Hatfield et al., 2008; Schildkopf et al., 2011). During senescence the damaged cells lose their replication potential because a so called „senescence-activated secretory phenotype" (SASP) will develop and cells will produce a number of cytokines and chemokines which induce inflammatory reactions. SASP development is stimulated by DNA damaging agents. It is well-known that ionizing radiation induces many types of DNA damages and by this manner SASP can be activated by radiation. It is however not clear whether SASP is immunogenic or tolerogenic. It seems if SASP is developing in tumour cells immediately after irradiation, the released inflammatory mediators induce immunogenic reactions. However, SASP developing in the microenvironment of tumours can initiate and maintain a chronic inflammatory reaction leading to tolerogenic immune suppressive effects (Davalos et al., 2010). These processes have been detected both in pre-cancerous and tumour cells (breast and prostate tumours, melanomas and mucosa hyperplasia) (Choi et al., 2000; Dhawan et al., 2000; Coppe et al., 2008a; 2008b).

As mentioned above, decreased expression of MHCI receptors on the surface of tumour cells is an important mechanism to bypass immune recognition. Thus DC cells that are primarily responsible for recognizing cells with altered antigenic structure are unable or less efficient to activate the immune system. It was shown in glioma, melanoma and colon carcinoma cell lines that ionizing radiation might increase MHCI expression helping antigen presentation (Hauser et al., 1993; Santin et al., 1996; Santin et al., 1997). In some cases even a long term effect lasting at least up to 11 days was detected (Reits et al., 2006). It is also important that not only single dose but fractionated irradiations might increase MHCI levels (Hauser et al., 1993). One of the potential explanation for the increased MHCI levels is that the interferon gamma (IFN-γ) production of tumour infiltrating cytotoxic T and NK cells present in the tumour microenvironment will be increased by radiation (Lugade et al., 2008). Reits et al. (2006) found that cytotoxic T cells will destroy more efficiently tumour cells with higher MHCI levels.

Beside MHCI receptors ionizing radiation can improve the cell surface concentration of other immune response regulating molecules, as well. Such molecules are for instance ICAM-1 (intercellular adhesion molecule type-1) or CD54 that has substantial role in the leukocyte infiltration of tumours. CD54 induction was reported in stomach, ovarium and colorectal tumours (Santin et al., 1996; Santin et al., 1997; Hareyama et al., 1998) after irradiation. It was also demonstrated that ionizing radiation can increase the amount of CD95 receptors on the surface of different tumour cells. CD95 is an important pro-apoptotic molecule, which induces apoptosis in the presence of functional p53 protein. Beside this CD95 can increase the cytotoxic effect of tumour infiltrating CD8+ T cells in a p53 independent manner. It was also suggested that ionizing radiation not necessarily increased CD95 cell surface levels, but the CD8+ T cell activating effect of existing CD95 receptors was improved (Sheard et al., 1999; Abdulkarim et al., 2002; Sheard et al., 2003; Park et al., 2003).

The NKG2D (Natural Killer Group 2D) receptor plays an important role in antitumour immunity. It modulates lymphocyte activation and promotes immunity to eliminate ligand-expressing cells. NKG2D ligands are produced by a number of cells including tumour cells.

NKG2D ligand production is induced by various stress factors including ionizing radiation. Recent data show that DNA damage which can be the result of radiation damages strongly affects NKG2D ligand productions, and immune activations (Gasser et al., 2005). Finally, ionizing radiation can increase the production of tumour-specific antigens (mucin-1, carcinoembryonic antigen) on cell surfaces (Garnett et al., 2004).

The tumour cells and almost all of the cells in the surrounding tumour microenvironment (lymphocytes, monocytes, macrophages, dendritic cells, endothel cells, fibroblasts, etc.) produce cytokines and chemokines. These molecules depending on their concentrations, on the producing and the target cells, might have either immune system activating or inhibiting effects. Ionizing radiation can influence these processes by several ways. It was mentioned formerly that ionizing radiation increases the IFN-γ production of melanoma cells (Lugade et al., 2008). It was also reported that radiation increased IL-1α, IL-6 and GM-CSF production in human lung carcinoma cells (Zhang et al., 1994), as well as IL-6 and IL-8 production in gliomas (Yamanaka et al., 1993). Yamamoto et al. (2003) compared the cytokine production of oral cavity epithelial carcinoma cells and healthy gum keratinocytes before and after radiation therapy. They found that the cytokine production (TNFα, TGFβ and GM-CSF) of tumour cells was higher compared to normal cells before irradiation. The IL-1β, IL-6, IL-10, TNFα and TGFβ production of tumour infiltrating lymphocytes was higher than the cytokine production of mononuclear cells present in peripheral blood. Ionizing radiation altered these profiles: the cytokine production of tumour cells decreased, while it was increased in tumour infiltrating lymphocytes. This is a very important proof that ionizing radiation can alter the profile and the concentration of cytokines capable to influence antitumour immunity. It was found in murine breast carcinoma cells that ionizing radiation increased CXCL6 chemokine production in tumour cells and as a result the number of tumour infiltrating lymphocytes was enhanced (Matsumura et al., 2008).

As can be seen above, ionizing radiation - beside improving the production of immune system activating cytokines – might also enhance the level of immune suppressing cytokines (IL-10, TGFβ and TNFα), contributing to general immune system suppression during tumour progression. While IL-10 is a definite immune suppressing agent, meanwhile TGFβ and TNFα might have dual effects depending on their level and on the tumour microenvironment. Ionizing radiation can strongly enhance TGFβ levels within tumours both by increasing directly TGFβ production and by promoting the conversion of the inactive form of TGFβ to an active one. This leads to the development and maintenance of a chronic inflammatory state that inhibits the function of DC cells and stimulates the conversion of CD4+ T cells to regulatory T cells (Barcellos-Hoff et al., 1994). IL-10 can be produced by several types of tumour cells such as oral cavity carcinomas (Yamamoto et al., 2003), certain melanomas, gastric carcinoma cells, non-small cell lung tumours, as well as by epithelial and basal cell skin carcinomas (Huang et al., 1995; Kim et al., 1995; Dummer et al., 1996; Morisaki et al., 1996). Beside tumour cells, tumour infiltrating immune cells, mainly monocytes and apoptotic T cells are the main sources of IL-10 production within tumours (Gao et al., 1998). Obviously ionizing radiation kills not only the tumour cells but induces the apoptosis of tumour infiltrating lymphocytes, contributing by this manner, too, to the increased production of IL-10.

Radiation effects on the immune system

As mentioned above, ionizing radiation can affect the immune system both by direct and indirect ways. The direct influence is through tumour infiltrating lymphocytes, macrophages and dendritic cells. It is usually a local effect. The indirect effect is mediated through the so-called immune-mediators produced by tumour cells and by healthy cells present in the tumour microenvironment. This influence might have both local and systemic consequences.

The radiation sensitivity of the immune system cells is very different. Lymphocytes and especially B cells and CD8+ cytotoxic T cells are extremely sensitive to radiation and die rapidly. Other lymphocyte subsets such as regulatory T cells and NK cells are relatively resistant to radiation. Dendritic cells and especially macrophages exhibit much higher radiation resistance than lymphocytes (Bogdándi et al., 2010). The varying radiation sensitivity of the cells in the tumour microenvironment might substantially modify anti-tumour immunity during radiation therapy. In the following subchapters we will summarize our knowledge on the radiation response of immune cells.

Dendritic cells (DC cells)

The dendritic cells are professional antigen presenting cells which can recognize the modified antigen structure of malignant cells therefore they are key elements of antitumour immunity. Malignant tissues contain only a limited amount of DCs and very frequently these DCs represent a subgroup which might induce immune suppression after meeting with tumour cell antigens (Sombroek, 2002; Norian et al., 2009). It was observed that certain tumours (breast, lung, pancreas, ovary tumour and melanoma) produce inflammatory mediators and growth factors (TGFβ, vascular endothelial growth factor or VEGF, cyclooxigenase-2 or COX-2) capable to inhibit the maturation and differentiation of DC cells to maintain an immune suppressive phenotype. It was also observed that these DC cells exhibit increased production of indoleamine-2,3-dioxygenase (IDO) enzyme, which can catalyse the oxidation of tryptophan which in turn will inhibit the cell division of effector T cells and can promote the activation of regulatory T cells (Munn et al., 2002).

It is obvious that the activation of tumour infiltrating dendritic cells is not sufficient alone to initiate an efficient anti-tumour immune attack. As mentioned before, large number of danger signals is released by dying tumour cells after irradiation. These signal molecules can efficiently activate DC cells through TLR4 and P2RX7 receptors. HMG1 – released by dying tumour cells - binds to TLR4, while P2RX7 is activated by free ATP (Ma et al., 2010b). TLR4 receptor activation initiates IL-1β production in DC cells, which then induce interferon gamma (IFNγ) production in cytotoxic CD8+ T cells. The importance of the functional TLR4 receptors in the development of an efficient anti-tumour immune attack is also proved by the finding that those breast tumour patients who had function loss resulting mutations in their TLR4 gene had shorter remission intervals after treatment than those who had functional TLR4 genes (Apetoh et al., 2007).

The way how tumour antigens are processed by DC cells and how they are presented to CD4 + and CD8 + T cells also determines whether immune stimulation or suppression is initiated.

It was observed that radiation improves the capacity of DCs to process antigens and to present them to T cells. Interestingly, this effect was reported in several independent studies, but none of the studies were able to prove that ionizing radiation increased the presence of activation markers on the surface of DC cells. However it was proved that radiation enhanced CD70 levels on DC cells which lead to the enhancement of T cell stimulation and increased IFNγ production in T cells (Liao et al., 2004; Huang et al., 2011; Burnette et al., 2011).

Another important step required for DC cells to trigger a tumour-specific immune response is to get to the regional lymph nodes and to present tumour antigens there to T lymphocytes. This process is helped with the expression of special, so-called „homing" receptors. One of these receptors is CCR7, which can recognize two ligands (CCL19 and CCL21). In collaboration these two ligands can activate CCR7 and initiate the transfer of DC cells to regional lymph nodes. There are reports that local irradiation can elevate CCR7 levels on DC cells and also the concentration of the special ligands in their neighbourhood (Cummings et al., 2012).

Tumour infiltrating macrophages (phagocytes)

Compared to DC cells the infiltration of tumours by macrophages is considerably higher, although obviously there are tumour specific differences: it is around 10-65%. Macrophages are usually present at higher number around necrotic regions and at the edges of tumours (Leek et al., 1999; Hashimoto et al., 2000). There are two distinct types of macrophages: M1 and M2; the former one is considered as an immune-stimulant, the other is an immune suppressor (Lewis et al., 2006). It is very important in anti-tumour immunity that which one is the dominant. M1 macrophages are capable to directly kill tumour cells; they can release immune-activating cytokines and activate antigen-presenting cells. They exhibit their cytotoxic effect either directly or through the release of nitrogen-monoxide (NO), or TNFα. TNFα production is helped by oxidative free radicals and it is less active in hypoxic regions (Naldini et al., 1994). Since radiation can decrease hypoxia through re-oxygenation and it also induces an oxidative stress, TNFα production is probably increased by irradiations. On the other hand nitrogen-monoxide is usually active in hypoxic regions. The activity of TNFα and nitrogen-monoxide changes with their local concentrations. At high concentrations both of them exhibit tumour cell killing, while low concentrations help tumour growth by inhibiting apoptosis, stimulating new blood vessel formation and suppressing anti-tumour immunity (Lee et al., 2002; Liao et al., 2007).

M2 macrophages besides inhibiting T cell immune responses, can promote blood vessel formations in tumours, and can stimulate proliferation and migration of tumour cells, as well. The development of M2 phenotype is helped by cytokines and chemokines, such as TGFβ, IL-10 and IL-4 produced by tumour cells. Later the M2 phenotype is self-maintained because macrophages are also producing TGFβ, IL-10 and IL-4. M2 macrophages can also release arginase that will block the immune-stimulating effect of M1 macrophages (Elgert et al., 1998).

As mentioned before macrophages are rather resistant to radiation, therefore they are not usually killed by radiation therapy. Radiation however can modify their phenotypes. Unfortunately, it is not really straightforward whether radiation can help cell differentiation toward the M1 or M2

phenotype. Both processes were observed in certain tumour models (Klug et al., 2013). Still, most of the publications point toward to the development of M2 phenotype. It was reported in a murine prostate cancer model that radiation induced arginase and nitrogen-monoxide production in tumour infiltrating macrophages. Prostate cancer cells exhibited higher proliferation capacity when they were co-cultured with these macrophages under *in vitro* conditions (Tsai et al., 2007). It was also shown that radiation resistance of tumours was in line with the extent of their infiltration with macrophages. The radiation sensitivity of murine B16 melanomas improved when macrophages were selectively removed from the tumour (Meng et al., 2010). Xu et al. reported that tumour infiltrating macrophages produced large amounts of colony stimulating factor 1 (CSF-1) both in a murine prostate tumour model and also in human prostate carcinoma patients. Improved radiation sensitivity was detected when this production was blocked by specific inhibitors (Xu et al., 2013).

T lymphocytes

The amount of tumour infiltrating lymphocytes might substantially contribute to the development of anti-tumour immunity and in the case of certain tumours might serve as an independent prognostic factor. However it seems that primarily not the exact number of the tumour infiltrating lymphocytes are important, rather their subtype will determine the efficiency of the anti-tumour attack. Usually an increased infiltration with CD8+ lymphocytes leads to a relatively good prognosis (Clemente et al., 1996; Curiel et al., 2004; Dahlin et al., 2011).

The invasion of lymphocytes into tumours is promoted by various soluble molecules (cytokines, chemokines, growth factors) produced by tumour cells and their micro-environment. Several publications demonstrated that radiation was influencing the phenotype of tumour infiltrating lymphocytes, but, just as in the case of macrophages, it was unclear whether this effect was beneficial or unfavourable to anti-tumour immunity. In a mouse B16 melanoma model Lugade et al. (2005) demonstrated that single dose and fractionated irradiations were able to increase tumour cell-specific T cell numbers within the tumour and also improved the migration of lymphocytes into the malignant tissues. Yasuda et al. (2011) treated a murine metastatic colorectal tumour by local irradiation of the primary tumour and by intra-tumour IL-2 injections and found that both the primary tumour and the liver metastases disappeared. Meanwhile, CD4+ effector T cell infiltration was strongly enhanced and regulator T cell presence was decreased in the tumours. This positive effect was detected only in the combined modality regime. This group has also reported that increased tumour infiltration with CD4+ and CD8+ T cells was a good prognostic factor for the beneficial response to chemo- and radiotherapies in human rectal tumour patients. In contrast, Quinfeng et al. (2013) investigated the infiltration of tumours with CD4+, CD8+ T cells and with Foxp3+ regulator T cells in cervix tumour patients before and after radiotherapy and detected that while levels of CD4 + and CD8 + T cells decreased, regulatory T cell levels did not change at all.

These controversial results suggest that the immunological characteristics of different tumour types are largely determined by the tissue microenvironment. The degree of tissue hypoxia, the intra-tumour pH, the structure of the tumour, and/or the cytokine milieu within the tumour are all contributing factors which fundamentally affect the local and/or systemic effects of radiotherapy on anticancer immunity.

CONCLUSIONS

It has been only very recently discovered that radiotherapy might have the ability to influence the immunological parameters of the tumours and the efficacy of immunotherapy in positive ways. Nowadays, both basic researchers and oncologists are becoming increasingly aware of the potential to combine immune- and radiation therapies. Still, we need significant advances in many areas to introduce this combined therapy in the daily routine of cancer treatments. Among others, we need better knowledge of the molecular and cellular processes through which radiation can influence anti-tumour immune responses. We should strongly increase the number of those clinical trials which investigate the combined effects of immune-, chemo- and radiation therapies. Last but not least, systemic predictive markers should be identified which allow the optimal tailoring of therapeutic combinations to the individual needs.

ACKNOWLEDGEMENTS

This work was supported by Hungarian NKFIH K124937 and VKSZ_14-1-2015-0021 grants.

REFERENCES

ABDULKARIM B, SABRI S, DEUTSCH E et al. (2002). Radiation-induced expression of functional Fas ligand in EBV-positive human nasopharyngeal carcinoma cells. Int. J. Cancer 86:229–237

APETOH L, GHIRINGHELLI F, TESNIERE A et al. (2007). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat. Med. 13:1050–1059

BARCELLOS-HOFF MH, DERYNCK R, TSANG ML et al. (1994). Transforming growth factor-beta activation in irradiated murine mammary gland J. Clin. Invest. 93:892–899

BOGDÁNDI EN, BALOGH A, FELGYINSZKI N et al. (2010). Effects of low-dose radiation on the immune system of mice after total-body irradiation. Radiat. Res. 174:480–489

BURNETTE BC, LIANG H, LEE Y et al. (2011). The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. Cancer Res. 71:2488–2496

CHOI J, SHENDRIK I, PEACOCKE M et al. (2000). Expression of senescence-associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia. Urology 56:160–166

CLEMENTE CG, MIHM MC JR, BUFALINO R et al. (1996). Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77:1303–1310

COPPE JP, PATIL CK, RODIER F et al. (2008a). Senescence-associated secretory phenotypes reveal cellnonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6:2853–2868

COPPE JP, BOYSEN M, SUN CH et al. (2008b). A role for fibroblasts in mediating the effects of tobacco-induced epithelial cell growth and invasion. Mol. Cancer Res. 6:1085–1098

COSIMI AB, BRUNSTETTER FH, KEMMERER WT et al. (1973). Cellular immune competence of breast cancer patients receiving radiotherapy. Arch. Surg. 107:531–535

CUMMINGS RJ, GERBER SA, JUDGE JL et al. (2012). Exposure to ionizing radiation induces the migration of cutaneous dendritic cells by a CCR7-dependent mechanism. J. Immunol. 189:4247–4257

CURIEL TJ, COUKOS G, ZOU L et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat. Med. 10:942–949, 2004.

DAHLIN AM, HENRIKSSON ML, VAN GUELPEN B et al. (2011). Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod. Pathol. 24:671–682

DAVALOS AR, COPPE JP, CAMPISI J et al. (2010). Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev. 29:273–283

DHAWAN P and RICHMOND A. (2002). Role of CXCL1 in tumorigenesis of melanoma. J. Leukoc. Biol. 72:9–18

DZHANDZHUGAZYAN KN, KIRKIN AF, THOR STRATEN P et al. (1998). Ecto-ATP diphosphohydrolase / CD39 is overexpressed in differentiated human melanomas. FEBS Lett. 430:227–230

DUMMER W, BASTIAN BC, ERNST N et al. (1996). Interleukin-10 production in malignant melanoma: preferential detection of IL-10-secreting tumor cells in metastatic lesions. Int. J. Cancer 66:607–610

ELGERT KD, ALLEVA DG, and MULLINS DW. (1998). Tumor-induced immune dysfunction: the macrophage connection. J. Leukoc. Biol. 64:275–290

GAO Y, HERNDON JM, ZHANG H et al. (1998). Antiinflammatory effects of CD95 ligand (FasL)-induced apoptosis. J. Exp. Med. 188:887–896

GARNETT CT, PALENA C, CHAKARBORTY M et al. (2004). Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. Cancer Res. 64:7985–7994

GASSER S, ORSULIC S, BROWN EJ et al. (2005). The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 436:1186–1190

HAREYAMA M, IMAI K, OOUCHI A et al. (1998). The effect of radiation on the expression of intercellular adhesion molecule-1 of human adenocarcinoma cells. Int. J. Radiat. Oncol. Biol. Phys. 40:691–696

HASHIMOTO I, KODAMA J, SEKI N et al. (2000). Macrophage infiltration and angiogenesis in endometrial cancer. Anticancer Res. 20:4853–4856

HATFIELD P, MERRICK AE, WEST E et al. (2008). Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. J. Immunother. 31:620–632

HAUSER SH, CALORINI L, WAZER DE et al. (1993). Radiation-enhanced expression of major histocompatibility complex class I antigen H-2Db in B16 melanoma cells. Cancer Res 53:1952–1955

HUANG M, WANG J, LEE P et al. (1995). Human non-small cell lung cancer cells express a type 2 cytokine pattern. Cancer Res 55:3847–3853

HUANG J, WANG QJ, YANG S et al. (2011). Irradiation enhances human T-cell function by upregulating CD70 expression on antigen-presenting cells in vitro. J Immunother 34:327–335

JAISWAL S, JAMIESON CH, PANG WW et al. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell 138:271–285

KIM J, MODLIN R, MAY RL et al. (1995). IL-10 production in cutaneous basal and squamous cell carcinomas. J Immunol 155:2240–2247

KLUG F, PRAKASH H, HUBER PE et al. (2013). Low-dose irradiation programs macrophage differentiation to an iNOS+/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 24:589-602

KÜNZLI BM, BERBERAT PO, GIESE T et al. (2007). Upregulation of CD39/NTPDases and P2 receptors in human pancreatic disease. Am J Physiol Gastrointest Liver Physiol 292:223–230

LEE VY, MCCLINTOCK DS, SANTORE MT et al. (2002). Hypoxia sensitizes cells to nitric oxide-induced apoptosis. J Biol Chem 277:16067–16074

LEEK RD, LANDERS RJ, HARRIS AL et al. (1999). Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. Br J Cancer 79:991–995

LEWIS CE, and POLLARD JW. (2006). Distinct role of macrophages in different tumor microenvironments. Cancer Res 66:605–612

LIAO YP, WANG CC, BUTTERFIELD LH et al. (2004). Ionizing radiation affects human MART-1 melanoma antigen processing and presentation by dendritic cells. J Immunol 173:2462–2469

LIAO YP, SCHAUE D, and MCBRIDE WH. (2007). Modification of the tumor microenvironment to enhance immunity. Front Biosci 12:3576–3600

LUGADE AA, MORAN JP, GERBER SA et al. (2005). Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol 174:7516–7523

LUGADE AA, SORENSEN EW, GERBER SA et al. (2008). Radiation-induced IFN-γ production within the tumor microenvironment influences antitumor immunity. J Immunol 180:3132–3139

LUMNICZKY K and SÁFRÁNY G. (2015). The impact of radiation therapy on the antitumor immunity: Local effects and systemic consequences. Cancer Lett 356:114-125

MA Y, KEPP O, GHIRINGHELLI F et al. (2010). Chemotherapy and radiotherapy: cryptic anticancer vaccines, Semin Immunol 22:113–124

MA Y, CONFORTI R, AYMERIC L et al. (2011). How to improve the immunogenicity of chemotherapy and radiotherapy, Cancer Metastasis Rev 30:71–82

MATSUMURA S, WANG B, KAWASHIMA N et al. (2008). Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol 181:3099–3107

MENG Y, BECKETT MA, LIANG H et al. (2010). Blockade of tumor necrosis factor alpha signaling in tumor associated macrophages as a radiosensitizing strategy. Cancer Res 70:1534–1543

MORISAKI T, KATANO M, IKUBO A et al. (1996). Immunosuppressive cytokines (IL-10, TGF-β) genes expression in human gastric carcinoma tissues. J Surg Oncol 63:234–239

MUNN DH, SHARMA MD, LEE JR et al. (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase, Science 297:1867–1870.

NALDINI A, CESARI S, BOCCI V. (1994). Effects of hypoxia on the cytotoxicity mediated by tumor necrosis factor-alpha. Lymphokine Cytokine Res 13:233–237

NORIAN LA, RODRIGUEZ PC, O'MARA LA et al. (2009). Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. Cancer Res 69:3086–3094

OBEID M, PANARETAKIS T, JOZA N et al. (2007). Calreticulin exposure is required for the immunogenicity of cirradiation and UVC light-induced apoptosis. Cell Death Differ 14:1848–1850

PARK IC, WOO SH, PARK MJ et al. (2003). Ionizing radiation and nitric oxide donor sensitize Fas-induced apoptosis via up-regulation of Fas in human cervical cancer cells. Oncol Rep 10:629–633

PEREZ CA, FU A, ONISHKO H et al. (2009). Radiation induces an antitumour immune response to mouse melanoma. Int J Radiat Biol 85:1126–1136

PRISE KM, and O'SULLIVAN JM. (2009). Radiation-induced bystander signalling in cancer therapy. Nat Rev Cancer 9:351–360

QINFENG S, DEPU W, XIAOFENG Y et al. (2013). In situ observation of the effects of local irradiation on cytotoxic and regulatory T lymphocytes in cervical cancer tissue. Radiat Res 179:584–589

REITS EA, HODGE JW, HERBERTS CA et al. (2006). Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy J Exp Med 203:1259–1271

SANTIN AD, ROSE GS, HISERODT JC et al. (1996). Effects of cytokines combined with high-dose gamma irradiation on the expression of major histocompatibility complex molecules and intercellular adhesion molecule-1 in human ovarian cancers Int J Cancer 65:688–694

SANTIN AD, HERMONAT PL, HISERODT JC et al. (1997). Effects of irradiation on the expression of major histocompatibility complex class I antigen and adhesion costimulation molecules ICAM-1 in human cervical cancer. Int J Radiat Oncol Biol Phys 39:737–742

SCHILDKOPF P, FREY B, OTT OJ et al. (2011). Radiation combined with hyperthermia induces HSP70-dependent maturation of dendritic cells and release of pro-inflammatory cytokines by dendritic cells and macrophages. Radiother Oncol 101:109–115

SHEARD MA, KRAMMER PZ, and ZALOUDIK J. (1999). Fractionated gamma-irradiation renders tumour cells more responsive to apoptotic signals through CD95. Br J Cancer 80:1689–1696

SHEARD MA, ULDRIJAN S, and VOJTESEK B. (2003). Role of p53 in regulating constitutive and X-radiation-inducible CD95 expression and function in carcinoma cells. Cancer Res 63:7176–7184

SOMBROEK CC. (2002). Prostanoids play a major role in the primary tumor-induced inhibition of dendritic cell differentiation. J Immunol 168:4333–4343

SOTO-PANTOJA DR, TERABE M, GHOSH A et al. (2014). CD47 in the tumor microenvironment limits cooperation between antitumor T-cell immunity and radiotherapy. Cancer Res 74:6771-6783

TSAI CS, CHEN FH, WANG CC et al. (2007). Macrophages from irradiated tumors express higher levels of iNOS, arginase-I and COX-2, and promote tumor growth Int J Radiat Oncol Biol Phys 68:499–507

XU J, ESCAMILLA J, MOK S et al. (2013). CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res 73:2782–2794

YAMAMOTO T, KIMURA T, UETA E et al. (2003). Characteristic cytokine generation patterns in cancer cells and infiltrating lymphocytes in oral squamous cell carcinomas and the influence of chemoradiation combined with immunotherapy on these patterns. Oncology 64:407–415

YAMANAKA R, TANAKA R, and YOSHIDA S. (1993). Effects of irradiation on cytokine production in glioma cell lines. Neurol Med Chir (Tokyo) 33:744–748

YASUDA K, NIREI T, SUNAMI E et al. (2011). Density of CD4(+) and CD8(+) T lymphocytes in biopsy samples can be a predictor of pathological response to chemoradiotherapy (CRT) for rectal cancer. Radiat Oncol 6:49

VERMEER DW, SPANOS WC, VERMEER PD, et al. (2013). Radiation-induced loss of cell surface CD47 enhances immune-mediated clearance of human papillomavirus-positive cancer. Int J Cancer 133:120–129

WILLINGHAM SB, VOLKMER JP, GENTLES AJ et al. (2012). The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci USA 109:6662–6667

ZHANG JS, NAKATSUGAWA S, NIWA O et al. (1994). Ionizing radiation induced IL-1α, IL-6 and GM-CSF production by human lung cancer cells. Chin Med J (Engl.) 107:653–657