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32 Inhibition of angiogenesis by antiinflammatory drugs

Masakazu Toi[1,2], Ryuichi Yoshida[1], Md. Zahidunnabi Dewan[3], Shoji Yamaoka[3] and Naoki Yamamoto[3]
[1]Department of Surgery, Faculty of Medicine, Kyoto University
Shogoin-Kawaharacho 54, Sakyo-ku, Kyoto, 606-8507, Japan, [2]Department of Surgery
Tokyo Metropolitan Cancer and Infectious Disease Centre, Komagome Hospital
3-18-22, Honkomagome Bunkyo-ku, Tokyo, 113-8677, Japan; [3]Department of
Molecular Virology, Graduate School, Tokyo Medical and Dental University
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

Abstract

Inflammation is one of major causes why cancers are possible to grow continuously. It provides multiple pro-survival signals for tumor cells. For instance, chemical mediators produced by the interaction between tumor cells and tumor stromal cells help to construct tumor neovasculatures. These stromal inflammatory cells are notably recruited from bone marrow so that tumors are utilizing the stem cell activity of bone marrow as a consequence. In order to block the pro-tumor relationship between tumor and bone marrow, the anti-inflammatory agents are important

Correspondence/Reprint request: Dr. Masakazu Toi, Department of Surgery, Faculty of Medicine, Kyoto University, Shogoin-Kawaharacho 54, Sakyo-ku, Kyoto, 606-8507, Japan. E-mail: toi@kuhp.kyoto-u.ac.jp

essentially. In particular inhibitors of nuclear factor (NF)-kB and those of cyclooxygenase (COX)-2 have been characterized to be promising at least in the experimental systems. In addition, it is indicated that the antitumor activities of conventional treatments such as chemotherapy are enhanced by the combination with anti-inflammatory agents. Therefore various types of inhibitors are tested in preclinical or clinical studies as a single treatment or combination. Although we need to resolve several issues for clinical application, it is important to investigate the anti-inflammatory agents for cancer treatment.

Introduction

Tumor angiogenesis is driven by oncogenic and inflammatory stimuli. Abnormality of oncogenes and tumor suppressor genes facilitates to induce angiogenic mediators continuously and tumor-associated inflammation enhances angiogenic reaction chronically. Although inflammatory reactions are complicated, abnormal accumulation of inflammatory cells such as macrophages and neutrophils are detected in most of human cancer tissues. Those inflammatory cells anchoring to tumor mircroenvironment are educated by cancer cells to produce pro-tumor molecules including angiogenic mediators. The inflammation tends to prolong unless original causes and cancer, are removed. Therefore, it is essentially important to control cancer-related inflammation, particularly angiogenic inflammation in order to suppress cancer progression.

Masters in angiogenesis

There are many direct and indirect evidences that show how inflammation is important for tumor angiogenesis [1]. Depletion of monocytic cells and macrophages results in the regression of tumors experimentally. The accumulation of tumor-associated macrophages (TAMs) and its activation correlate significantly with the increase in tumor microvessel density and poor prognosis in various types of human cancers [2-4]. Anti-inflammatory agents such as cyclooxygenase (COX)-2 inhibitors are able to suppress neovascularization. [5] It is clear that inflammation plays a crucial role in determining the phenotype of tumor angiogenesis and of tumor progression.

Recent studies have indicated that there are two types of angiogenic stimuli, consisting of oncogenic stimuli based on the disorders of oncogenes and tumor suppressor genes and inflammatory stimuli mainly due to the interaction between tumor cells and stromal cells, in tumor angiogenesis [6,7]. Genomic abnormalities such as overexpression of Her-2 and ras mutation help to produce pro-angiogenic molecules such as vascular endothelial growth factor (VEGF) and angiopoietins (ANGs) constitutively [8]. On the other hand, negative regulators such as thrombospondins and soluble VEGF receptor-1 (sVEGFR1) tend to be down-regulated by oncogenic disorders, suggesting the balance between pro-angiogenic regulators and anti-angiogenic regulators is shifted to the relative dominance for pro-angiogenic side [9]. This concept has been postulated by Folkman et al. initially and it has been accepted widely [10]. In sense, it has been demonstrated that suppression of oncogenic stimuli, such as inhibition of Her-2 signals by anti-Her-2 monoclonal treatment, results in reducing pro-angiogenic molecules like VEGF and in tumor regression associated with elimination of tumor vasculatures [11]. In addition to anti-Her therapy, various types of inhibitors for oncogenic growth factor signals are developed clinically or preclinically so that it is crucial to suppress

inflammatory stimuli as well as oncogenic stimuli in order to control tumor angiogenesis more efficiently. This idea is still hypothetical but it should be investigated in further depth.

Hypoxia-inducible factor (HIF) family is thought to play a pivotal role in the oncogenic pathways (Figure 1). Notably HIF-axis is a central player for hypoxia-induced angiogenesis [12,13]. This transcription factor axis is responsible for not only VEGF family but also stromal-derived cell factor (SDF)-1/CXCR-4, which is known to be a crucial system in angiogenesis and metastasis. It was recently documented that von Hippel-Lindau tumour suppressor protein pVHL negatively regulates CXCR4 expression owing to its capacity to target HIF for degradation under normoxic conditions however this process was suppressed under hypoxic conditions. SDF-1 gene expression is also regulated by HIF-1 [14]. It is reported that expression of SDF-1 was induced in *in vivo* ischemic tissue in direct proportion to reduced oxygen tension. Thus, SDF-1 and CXCR4 activation depends upon HIF activation [15]. HIF-1α protein synthesis is regulated by activation of the phosphatidylinositol 3-kinase (PI3K) and ERK mitogen-activated protein kinase (MAPK) pathways [16]. These pathways can be activated by signaling via receptor tyrosine kinases or G-protein-coupled receptors. Degradation of HIF-1α protein is regulated by O_2-dependent prolyl hydroxylation that targets the protein for ubiquitylation.

It is intriguing to ask what happens on tumor angiogenesis if the HIF-axis is blocked. A recent investigation by Mizukami et al provided some hints to answer this question. It was showed that HIF-1α knockdown was able to reduce tumor growth, presumably due to down-regulation of tumor cell proliferation, whereas it was unable to eliminate microvessel density in xenograft models of human colon cancer [17]. A significant but partial reduction in VEGF expression in tumor tissues was found, and interestingly, in contrast to VEGF, IL-8 expression was up-regulated in concert with enhanced activation

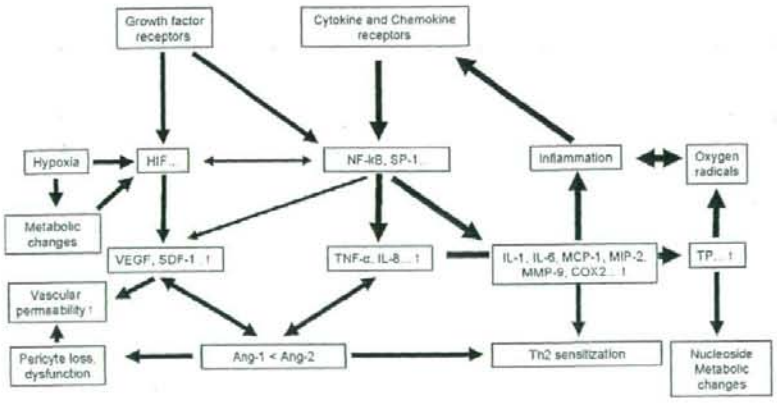

Figure 1. Possible cascades of angiogenesis stimuli consisted of oncogenic stimuli and inflammatory stimuli: Relationship between hypoxia-inducible factor-axis and nuclear factor-kB-axis.

of nuclear factor (NF)-*k*B, speculating that IL-8 might compensate VEGF function for angiogenesis. NF-*k*B axis may play a reciprocal role for HIF-axis in certain conditions. It has been reported that HIF-1 protein is constitutively expressed in various types of human tumor tissues and these expressions correlated with poor prognosis significantly [18]. Nevertheless, it is also true that the tumor lacking a constitutive HIF expression does not necessarily mean less angiogenic, so that another side of question, what happens on HIF-axis by inhibition of NF-*k*B-axis, could be important to consider [19]. At present, the information is limited but several studies have shown that inhibition of NF-*k*B resulted in down-regulation of VEGF in breast cancer cells and head and neck cancer cells. Similarly, genetic or pharmacologic blocking of NF-*k*B suppressed CXCR4 induction in breast cancer cells [20]. For the therapeutic application, double blockade of HIF-axis and NF-kB-axis seems to be reasonable. In fact, a dual inhibition of VEGF and IL-8 achieved an additive or synergistic effect on tumor regression experimentally.

Tumor-associated macrophages

Solid tumors provide numbers of machineries to evade immune surveillance system. One important mechanism is to educate tumor-associated macrophages (TAMs). Although precise mechanisms are still largely unknown, TAMs are likely to work for tumor progression. In our previous studies, it was revealed that the activated TAMs such as thymidine phosphorylase (TP) positive and CD-68 positive macrophages are associated with unfavorable prognosis [21]. On the contrary, TP-negative TAMs rather correlate with favorable prognosis. TP is a nucleoside metabolism enzyme with multiple activities including stimulation of endothelial chemotaxis. Various cytokines such as TNF-α

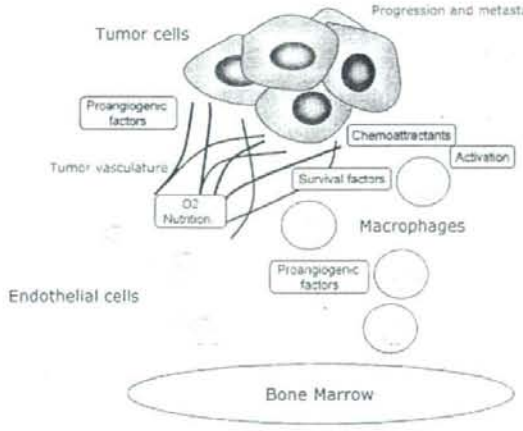

Figure 2. Relationship between tumor cells and bone marrow: It looks that the tumor cells utilize the bone marrow for their endless growth by educating bone marrow-derived cells.

and IL-1 and are capable of inducing TP. The concept that the activated TAMs have a pro-tumor property has been also demonstrated by other investigations with different markers like heme oxygenase-1 and Cap43 [22-24]. The activated TAMs can produce large amounts of chemical mediators and proteases that help tumor angiogenesis and tumor cell invasion.

Unless the basal cause and cancer are removed, activated inflammation is likely to continue because originally it is a host defense mechanism. These chronic inflammation functions as a positive feedback loop between tumor cells and bone marrow. Various types of cytokines and chemokines such as IL-1 and macrophage chemoattractant protein (MCP)-1 are known to play a key role for recruiting TAMs from bone marrow and its activation [25]. In addition, tumors request bone marrow to provide progenitor endothelial cells. VEGF, and other mediators like SDF-1 and MCP-1 can be participating in the recruitment of these progenitor cells into the tumor bed. Mobilization of the progenitor cells may also prepare the condition for tumor metastasis [27-29]. Tumor utilizes the bone marrow stem cells to progress (Figure 2). Blocking of the relationship between tumor and bone marrow may result in diminishing systemic spread of tumor cells.

Anti-inflammatory agents

NF-*k*B inhibitors

The nuclear factor-kB (NF-kB) proteins are a small group of closely related transcription factors, which in mammals consist of five members: Rel (also known as c-Rel), RelA (also known as p65 and NF-kB3), RelB, NF-kB1 (also known as p50) and NF-kB2 (also known as p52.) In resting cells, NF-κB is sequestered as an inactive precursor in association with inhibitory IκBs in the cytoplasm [30,31]. On stimulation (virus, bacteria, TNF-a and IL-1), Ikbs are rapidly phosphorylated, ubiquitinated, and degraded by a proteasome-dependent pathway allowing active NF-κB to translocate into the nucleus where it can activate the expression of a number of genes. NF-κB activation has been connected with multiple processes of oncogenesis including control of apoptosis, cell-cycle, differentiation, and cell migration. A major link between inflammation and carcinogenesis may depend on NF-κB activation. It was estimated that inflammation may play a role in the etiology of 15% human cancers, mostly acting as a tumor promoter. Furthermore, it is important to note that NF-*k*B is induced by anti-cancer treatments. It potentially acts for therapeutic resistance. In a recent study of preoperative chemotherapy for primary breast cancer, it was unlikely to achieve pathological complete response for tumors with NF-*k*B expression, despite of partial response was possible to achieve. Right now, major hurdle to treat cancer patients is to develop of drug-resistance of cancer cells as well as side effects of drugs using conventional therapies. Therefore, development of new and effective therapeutic strategies is urgently needed to improve the prognosis of cancer and viral infected patients. Recently, treatment strategies to target molecules responsible for the maintenance and growth of the tumor cells have been emphasized [32-37]. These strategies intensify the specificity of the treatment and minimize undesirable toxicity to normal cells. Thus, the molecular target strategy provides an opportunity to overcome resistance to conventional chemotherapy [34]. Despite the diversity in clinical manifestations

Table I. NF-κB inhibitory agents.

Inhibitors	*Dietary agents*
Bcl2 inhibitors	Curcumin (Tumeric curry)
DHMEQ	Epigallocatechin-3-gallate
EGF receptor related protein	(Green tea)
Flavopiridol	Human milk
HDAC inhibitors	Genistein (Soybean)
HIV protease inhibitors	Lupeol (Olive. Mango)
HSP90 inhibitors	Lycopene (Tomato)
Proteinase inhibitors	Resveratol (Red grapes)
Proteosome inhibitors	Sesquiterpene lactone
Synthetic retinoids	(Asteracease plants)
NSAIDs	Others
Steroids	
Other IKK modulators/ inhibitors	

of cancers, strong and constitutive NF-kB activity was reported to be a unique and common characteristic of many cancer cells that triggers proliferation and expression of various cytokines, and by which these cells were prevented from undergoing apoptosis. Therefore, targeting the NF-kB pathway and inhibition of NF-kB activity is a logical molecular target strategy for cancer therapy. Bay 11-7082, a specific and effective NF-kB inhibitor, suppressed the NF-kB activity of adult T-cell leukemia (ATL) cells and prevented the tumor growth and infiltration of ATL cells in murine model [35]. Bay 11-7082 and Bay 11-7085 induced apoptosis of colon cancer cells.Treatment of immunocompromised mice with Bay 11-7085 completely inhibited tumor implantation in the liver after the intraperitoneal delivery of HT-29 colon cancer cells. DHMEQ, a novel NF-kB inhibitor selectively targets constitutive NF-kB activity and induces apoptosis of ATL, multiple myeloma, Hodgking's lymphoma, breast cancer, and prostate cancer cells in vitro and in vivo [38-41]. Ritonavir, an HIV protease inhibitor very efficiently prevented tumor growth and leukemic infiltration of ATL cells at the same dose used for treatment of patients with AIDS [42]. Proteasome inhibitors inhibited the Ikb degradation, which lead to the maintenance of NF-kB in the cytoplasm that has shown promising anticancer responses both in vitro and in vivo [43,44]. A novel NF-kB inhibitor, IMD-0345 inhibits the NF-kB activity and induces the apoptosis of Neoplastic mast cells and breast cancer cells [45]. These findings suggest that NF-kB inhibitor may be a promising compound that targets constitutively activated NF-kB in cancer and virus infected cells and that can translate this strategy into clinical medicine. These results would provide a new concept and novel platform for development of new drugs against cancer. In addition, dietary agents that have anti-NF-kB activities have potential for cancer prevention [34,46-48].

COX-2 inhibitors

Prostaglandin endperoxide synthase, commonly called cyclooxygenase (COX) has two known isoforms namely COX-1 and COX-2. COX-1 is constitutively expressed in almost all normal tissues, and is responsible for regulation of 'housekeeping' functions. COX-2, an inducible prostaglandin synthase that catalyzes the formation of

prostaglandins and other eicosaniods from arachidonic acid. COX-2 protein mainly mediates a pro-inflammatory role and is overexpressed in several human malignancies such as colorectal cancer and adenoma, gastric cancer, breast cancer and brain tumor. Furthermore, COX-2 has been found to play important roles in cell adhesion and migration, apoptosis and angiogenesis through animal models. Non-steroidal anti-inflammatory drugs (NSAIDs), including COX2 inhibitors, are well known for the regression of adenomatous polyps of the colon and preventing the development of various cancers. The most commonly accepted theory to account for the inhibitory effects of these agents on the inflammatory response and their ability to prevent cancer arises from the idea that inhibition of cyclooxygenase activity by NSAIDs prevents prostaglandins synthesis. The prevention of prostaglandin synthesis by inhibition of COX2 activity therefore potentially suppresses mitogenic signaling, angiogenesis, tumor-cell invasion and metastasis. Another possible mechanism is that NSAIDs could also inhibit the activation of NF-kB. Salicylates (NSAIDs such as aspirin) inhibit the activity of IKKb and prevent NF-kB activation. Sulindac, an NSAID that induces regression of adenomas in patients with familial adenomateous polyposis, inhibits NF-kB pathway by inhibiting IKK activity [49]. NF-kB has been reported to regulate the COX2 promoter, leading to transcriptional activation of the COX2 gene [50]. Recent studies indicate that the COX2 inhibitor celecoxib inhibits NF-kB activation through inhibition of IKK and AKT activation, leading to down-regulation of COX2 synthesis [51]. Therefore, a COX2 inhibitor would seem to be a possible candidate for inhibition of NF-kB, leading to increase sensitivity of cancer cells to anticancer drugs. Sulfasalazine, an anti-inflammatory and immunosuppressive agent inhibits IKKa and IKKb, leading to the inhibition of NF-kB might be a useful complementary therapies for cancers with constitutively active NF-kB [52].

CXCR4 antagonists

Chemokines and their receptor have recently received considerable attention because of their important role in immune and inflammatory responses, angiogenesis, hematopoiesis, cancer, and HIV-1 infection [53-59]. Muller et al. first reported that chemokine receptor CXCR4 is highly expressed in human breast cancer cells, malignant breast tumors and metastatic tumors [60]. CXCR4-expressing breast cancer cells aggressively metastasize in secondary organs, where SFD-1 expression is significantly higher. Neutralization of SDF-1/CXCR4 interaction significantly inhibits the metastasis of breast cancer cells in distant organs. Systemic administration of the CXCR4 antagonist AMD3100 inhibited the growth of intracranial glioblastoma and medulloblastoma xenografts, and increased tumor cell apoptosis within 24 hours [61]. It appears that CXCR4 signaling may promote breast cancer through a wide range of mechanisms, including proliferation and survival of cancer cells, angiogenesis, and chemoinvasion of cells at primary and metastasis sites. Recent studies using mouse models of breast cancer and specimens from human tumors emphasize the importance of the tumor microenvironment in controlling the SDF-1/CXCR4 signaling pathway. CXCR4 antagonist KRH-1636 (12 mg/kg/day) inhibited tumor growth at the sites of the primary region and metastasis to lung in our murine model (unpublished data). The comprehensive study of chemokines and receptors in primary tumors, metastatic lesions and corresponding normal tissues will be crucial to further understanding of the cancer chemokine network. Therefore, CXCR4 is a potential therapeutic target in human cancer,

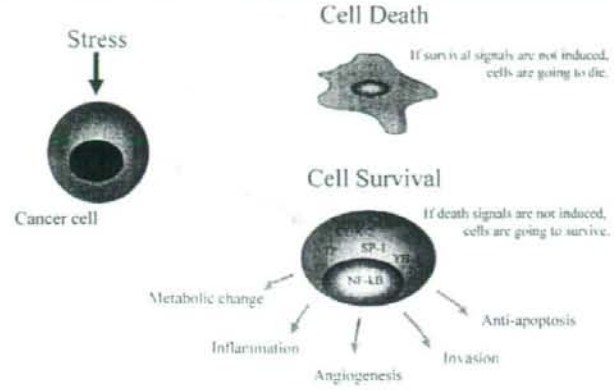

Figure 3. Stress including anticancer treatments can induce cell survival signals such as nuclear factor-kB, cyclooxygenase-2 and thymidine phosphorylase those are involved in inflammatory angiogenesis closely.

although more extensive studies using established tumors and antagonists of other receptors are required.

Future perspectives

Anti-inflammatory agents are important to incorporate into cancer therapy, because it is evident that chronic inflammation is a crucial mechanism to support an endless progression of cancer. Inflammatory cells and progenitor cells derived from bone marrow are mobilized and recruited to tumor bed by inflammatory mediators directly or indirectly, and then these cells are likely to function for produce pro-tumor stroma including tumor vasculatures. In the experimental studies or in the suppression of in situ diseases like adenomatous polyps, the anti-inflammatory agents can suppress neovascularization and tumor growth. Nevertheless, it is still unclear that anti-inflammatory agents are able to regulate cancer growth or not. For the clinical application, in particular for the treatment of invasive cancers, it might be important to consider the anti-immune aspect of anti-inflammatory agents. In recent clinical observation of rheumatoid arthritis, it seems that anti-TNF therapy tends to increase the incidence of cancer. Thereby, it would be essential to maintain anti-immune activities of the host cells during the treatment with anti-inflammatory agents. Furthermore, it is warranted to predict or prevent anti-cardiovascular side effects of anti-inflammatory drugs especially COX-2 inhibitors. However, theoretically and conceptually, it is reasonable to develop anti-inflammatory agents for cancer treatment and prevention. Since we have learned several important lessons from the clinical trials with COX-2 inhibitors and other anti-inflammatory agents, it is required to consider novel ideas and approaches to overcome the negative aspects of anti-inflammatory agents and to develop it for cancer patients or cancer high-risk individuals.

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Therefore, is EGFR targeting in pancreatic cancer a futile research area, especially when monoclonal antibodies are used for targeting? In my opinion the answer to this question is probably no. Research on the identification of predictive biomarkers is underway, and, therefore, EGFR blockade with monoclonal antibodies will probably be revisited in the near future when these studies are successfully completed. Moreover, given the redundancies of signalling pathways, EGFR inhibitors might have greater benefits when combined with other targeted treatments. Preclinical models of pancreatic cancer should guide our study of targeted combinations in clinical trials and help refine the identification of biomarkers. Finally, EGFR blockade might have greater benefit when used alone or in combination with different cytotoxic drugs, because gemcitabine alone offers only a small clinical benefit to patients.

The tradition of randomly combining drugs in the hope of achieving a better outcome in an unselected patient population or patients selected on unproven scientific grounds should be replaced by individualised treatments that have a solid scientific rationale. An exploration of predictive biomarkers should start before and continue alongside properly designed clinical trials. In this way, futile and costly exercises are avoided and patients with advanced pancreatic cancer might have the active treatments they so desperately need.

Philip A Philip
Karmanos Cancer Institute, Detroit, MI, USA
philipp@karmanos.org

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Long-term outcomes of aromatase inhibition for breast cancer

See Articles page 45

Hormone manipulation is an essential treatment option for breast cancer. Oestrogen depletion decreases disease occurrence and suppresses disease progression, especially hormone-dependent growth and metastasis. Recent accumulated data from postoperative adjuvant trials that compared use of third-generation steroidal or non-steroidal aromatase inhibitors with tamoxifen (a selective oestrogen-receptor modulator) have suggested that aromatase inhibitors are better than tamoxifen at decreasing disease recurrence and occurrence of contralateral breast cancer, as a class effect.[1–3]

Major guidelines and consensus meetings recommend 5-years of adjuvant treatment with aromatase inhibitors or 2–3 years of tamoxifen followed by 2–3 years of aromatase inhibitors as a standard treatment for women with breast cancer who are hormone-receptor-positive and postmenopausal.[4,5] However, the long-term therapeutic effect of treatment with aromatase inhibitors, especially disease control, after cessation of the treatment and adverse effects on bone and the cardiovascular system, have yet to be clarified.

In this issue of *The Lancet Oncology*, the Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group report findings of an analysis of 100-month follow-up data.[6] In the overall intention-to-treat and hormone-receptor-positive populations, disease-free survival, time to recurrence, time to distant recurrence, and incidence of new contralateral breast cancer improved significantly in women assigned anastrozole compared with women assigned tamoxifen. This improvement in disease control with anastrozole treatment was maintained for more than 8 years, suggesting that the therapeutic effect of 5 years'

treatment with aromatase inhibitors can be prolonged for over 3 years after treatment cessation. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) showed that disease control by use of 5 years' treatment with tamoxifen is maintained after cessation of treatment for 5–10 years.[7] Therefore, given the long-term findings from the ATAC trial reported in this issue,[6] anastrozole might have a larger carryover effect after cessation of treatment than tamoxifen, which should be taken into consideration in clinical-practice decisions. Likewise, a lower number of recurrences and of new contralateral breast cancers after anastrozole compared with tamoxifen was also maintained in the latest findings from the ATAC trial.

Despite the findings mentioned above, no significant survival advantage for anastrozole over tamoxifen was shown in this trial.[1,6] Deaths after recurrence were fewer in women assigned anastrozole than in those assigned tamoxifen (350 *vs* 382); however, deaths without recurrence were more frequent with anastrozole treatment than for tamoxifen (279 *vs* 242). The researchers did not note any significant difference in the incidence of deaths due to cardiovascular or cerebrovascular disease, but deaths due to second primary non-breast cancers and deaths due to other causes were more frequent in patients assigned anastrozole. Endometrial cancers, ovarian cancers, and melanomas were less frequent in those assigned anastrozole; however, colorectal cancers, lung cancers, and head and neck cancers were more frequent in women assigned anastrozole. With the exception of endometrial cancers, no statistically significant difference was noted for the occurrences of these cancers.

According to epidemiological studies, hormone-replacement treatment probably decreases the risk of developing colorectal cancer.[9] Additionally, oestrogen receptors α and β have been shown to inhibit the development of adenomatous polyposis coli (*APC*)-dependent colon cancer in mice.[10] By contrast, aromatase seems to enhance disease progression in lung cancers,[11] suggesting that the role of oestradiol in tumour progression or tumour regression could be diverse and dependent on cancer type. Since oestrogen receptor β is widely expressed in many organs, the effects of hormone manipulation on non-breast malignant disease occurrence needs to be assessed further.

Furthermore, in two trials that studied treatment with tamoxifen followed by aromatase inhibitors (such as exemestane and anastrozole), despite relatively short follow-up, incidence of second primary non-breast cancer was lower in patients assigned an aromatase inhibitor than in those assigned tamoxifen alone.[2,3] Therefore, we might not need to worry at present about the increased numbers of colorectal, lung, and head and neck cancers noted in the long-term ATAC findings for patients assigned anastrozole, but we need to continue collecting data on the incidence of these second primary non-breast cancers.

Fracture incidence is one of the adverse effects of aromatase inhibition that has caused most concern for oncologists and patients. Previous findings from both the ATAC group and others have shown that during the treatment period, patients assigned to an aromatase inhibitor have a higher incidence of bone fracture than those assigned to tamoxifen.[6,12] However, in the post-treatment period, the incidence of fractures decreased in both groups of patients,[6] and the difference in fractures between the two treatment groups was no longer apparent.[6] These findings lend support to a working hypothesis that bone damage by aromatase inhibition is reversible and potentially manageable, although the mechanisms of action of the bone damage by aromatase inhibitors and subsequent apparent recovery are still unclear.[13]

Oestrogen blockade is a core concept in the management of hormone-receptor-positive breast cancers. The ATAC trial has elucidated that aromatase inhibition can achieve a larger carryover effect in long-term disease control compared with tamoxifen treatment. However, we still need to pay attention to long-term follow-up findings, not only of this trial, but also of other trials containing aromatase inhibitors because an advantage in terms of overall survival has not yet been confirmed.

Masakazu Toi
Breast Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
toi@kuhp.kyoto-u.ac.jp

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In 2007 *The Lancet Oncology* published themed issues on paediatric oncology and on breast cancer. We will be continuing this concept into 2008 with a themed issue on lung cancer. Despite many advances in treatment over recent years, lung cancer is still the number one cause of death due to cancer in the world[1] and mean relative 5-year survival is only 12·6% in Europe[2]. *The Lancet Oncology* is therefore issuing a call for papers that report on major advances in the management of lung cancer. In particular, we are interested in the results of phase III randomised clinical trials. Accepted papers will be published in *The Lancet Oncology* to coincide with the International Lung Cancer Conference ([ILCC] Liverpool, UK, July 9–12, 2008). We are especially interested in research that will be presented at this conference, but we will also consider other suitable articles. If your study describes, in part or wholly, a study accepted for presentation at the ILCC, please let us know the precise details of the type of presentation (such as poster or oral presentation), including dates and times, so that publication in *The Lancet Oncology* can be scheduled to comply with ILCC's embargo policies. Articles should be submitted via *The Lancet Oncology*'s online submission service, and all authors must clearly state in the covering letter that their submission is in response to the "Lung Cancer Call for Papers". The deadline for submissions is May 2, 2008.

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Lidia Siemaszkiewicz
The Lancet Oncology, London NW1 7BY, UK

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Erratum

Bueno-de-Mesquita J, van Harten W H, Retel V P, et al. *Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER).* Lancet Oncol 2007; **8:** 1079–87. The last two sentences of the Summary's Findings should have read 'St Gallen guidelines identified 353 (83%) patients with poor prognosis and discordance with the signature in 168 (39%) patients. Nottingham Prognostic Index recorded 179 (42%) patients with poor prognosis and discordance with the signature in 117 (27%) patients.'

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Molecular target therapy: basics and clinical application

Shinzaburo Noguchi · Masakazu Toi

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Breast cancer is unique in that molecular target therapy has been widely practiced and has been playing a very important role in its treatment, i.e., hormonal therapy with tamoxifen can be considered as one of the oldest molecular target therapies. Tamoxifen selectively binds to estrogen receptor (ER) and antagonizes the estrogen-dependent growth-stimulative effects. Tamoxifen has been widely used for the treatment of breast cancer in the metastatic and adjuvant setting as a golden standard for more than two decades. Hormonal therapy with aromatase inhibitor is also a molecular targeted therapy because the third-generation aromatase inhibitors selectively bind to aromatase and inhibit its action, resulting in deprivation of intra-tumoral estrogens.

Another example of molecular target therapy for breast cancer is trastuzumab (humanized anti-HER2 antibody). Identification of *HER2* gene amplification in about 20% of breast cancers leads to trastuzumab, which is currently used as a standard treatment not only in the metastatic setting but also in the adjuvant setting. Furthermore, recent studies have revealed that trastuzumab plus chemotherapy (taxane and anthracycline) dramatically increases the complete pathological response in the neoadjuvant setting, prompting the future use of this combination in this setting. Other novel targeted treatments which are under clinical evaluation, including antiangiogenic compounds (bevacizumab, sunitinib, and others) and bi-functional drugs such as lapatinib [anti-HER2 and (epidermal growth factor receptor) EGFR agent] are showing promise.

At the 15th Annual Meeting of the Japanese Breast Cancer Society in Yokohama, Symposium 3, held on 30 June 2007, was entitled "Molecular target therapy: basics and clinical application". In that symposium, updated results of recent clinical studies on the development of molecular target therapies in breast cancer as well as challenges to design a drug in silico and to develop a new drug delivery system targeted at hypoxia were presented by seven speakers, including one invited from the United States. Summaries of their remarks follow.

The first speaker Dr. Kathy D. Miller from the Breast Care and Research Center, Division of Hematology and Oncology, Indiana University School of Medicine, USA, made a keynote address on molecular target therapy. She talked about the history of molecular target therapies and stressed the important role played by basic research in the development of such therapies. Updated results of pivotal clinical trials on trastuzumab, lapatinib, and sunitinib were also presented with great enthusiasm. Trastuzumab has been shown to be very active not only in the metastatic setting but also in the adjuvant setting, and adjuvant trastuzumab is now well accepted as a standard therapy for HER2-positive breast cancer. Furthermore, recent success in the introduction of trastuzumab in the neoadjuvant setting in combination with paclitaxel and 5-fluorouracil, epirubicin, cyclophosphamide (FEC) results in a surprisingly high pathological complete response rate with no serious cardiotoxicity, making further studies of neoadjuvant trastuzumab justifiable and attractive. Lapatinib, a dual inhibitor of EGFR and HER2, has also been shown to be active in the metastatic setting for HER2-positive breast cancer which becomes resistant to trastuzumab therapy, and the combination of lapatinib with capecitabin has been

S. Noguchi (✉)
Department of Breast and Endocrine Surgery,
Osaka University Graduate School of Medicine, Osaka, Japan
e-mail: noguchi@onsurg.med.osaka-u.ac.jp

M. Toi
Department of Breast Surgery, Kyoto University Graduate
School of Medicine, Kyoto, Japan

demonstrated to be better than capecitabin alone in terms of time to progression (TTP). Sunitinib, an antiangiogenic compound, has been reported to show a promising efficacy in heavily pretreated metastatic breast cancer. Dr. Miller is an author of one of the articles in this issue, in which more detailed information is available.

Dr. Reiki Nishimura from Kumamoto City Hospital talked about his group's data on treatment with trastuzumab in metastatic breast cancer patients with special reference to the relevance of trastuzumab monotherapy, although trastuzumab is usually given concurrently with chemotherapy. He stated that a combination of trastuzumab and chemotherapy brings about a better response rate than trastuzumab monotherapy, but survival duration was statistically not different, and he concluded that "trastuzumab monotherapy can be considered an option for patients with non-life-threatening disease". More in-depth information from Dr. Nishimura's presentation is contained in an article in this issue.

Dr. Hiroji Iwata from the Aichi Cancer Institute Hospital, representing the Lapatinib Study Group, made a presentation about the phase II study on lapatinib that was conducted in Japan, targeting HER2-positive breast cancer pretreated with anthracycline, taxane, and trastuzumab. The response rate to lapatinib was 36%, with a median TTP of 3.8 months. Although the group studied the association between response to lapatinib, and expression and mutation of various markers (EGFR, HER2, PIK3CA, PTEN), they found no significant association. A further study is in progress.

Dr. Daishu Miura from the Toranomon Hospital studied the mechanism of the additive effect of trastuzumab with paclitaxel from the viewpoint of antibody-dependent cellular cytotoxicity (ADCC) through determination of ADCC before and after trastuzumab monotherapy or combination therapy with paclitaxel. He suggested the possibility that the additive effect of trastuzumab and paclitaxel can be partially explained by the increase in ADCC, which is induced by this combination therapy.

Dr. Hiroo Nakajima from the Kyoto Prefectural University of Medicine introduced a novel in silico-designed HER2-targeting agent, HER2 reactive peptide-self-assembling regulatory molecule (HRAP-SARM), which binds to HER2 hot spots and blocks heterodimerization of HER family proteins such as HER2/HER3 dimers. Experimentally, the data look promising. These unique ideas may provide a new paradigm of individualized therapy for breast cancer particularly for HER family relevant cancers. Additional information on the subject of Dr. Nakajima's presentation appears in an article in this issue.

Dr. Teruhiko Fujii from Kurume University focused on the significance of Cap43 and YB-1 in hormone-dependent and -independent growth of breast cancer. An antimetastatic gene *Cap43* expression was down-regulated by estrogen but upregulated by tamoxifen in hormone-dependent breast cancer cells. YB-1, a stress-inducible transcription factor, is known to contribute to cell survival. Interestingly, nuclear translocation of YB-1 was associated significantly with an unfavorable prognosis of primary breast cancer patients. He suggested that these molecules could be new targets in breast cancer treatment. More detailed information on the subject of Dr. Fujii's presentation appears in an article in this issue.

Dr. Minoru Fujimori from Shinshuu University demonstrated the hypoxia-targeting drug-delivery system APS001. APS001 contains the nonpathogenic anaerobic bacterium *Bifidobacterium longum* transfected with cytosine deaminase (CD), which converts 5FC– 5FU. It is possible to achieve a selective high concentration of 5FU in a tumor microenvironment under hypoxic conditions when 5FC is administered simultaneously. He indicated that these approaches are effective experimentally, and several clinical trials are planned currently.

Recent development of genome-wide studies including gene-expression profiling and copy-number analysis has enabled the detection of tumor-specific genetic changes such as up-regulation or down-regulation of gene expression or gene amplification or deletion with high efficiency. Such tumor-specific genetic changes have the potential to become targets for the development of therapy. Therefore, persistent and relevant basic research to identify the key molecular alterations, which promote breast cancer growth, is of vital importance.

Round Table Meeting

細胞死

司会
京都大学大学院医学研究科外科学講座乳腺外科学教授
戸井　雅和
Masakazu Toi

慶應義塾大学医学部先端医科学研究所
遺伝子制御研究部門教授
佐谷　秀行
Hideyuki Saya

東北大学大学院医学系研究科医科学専攻
病理病態学講座教授
笹野　公伸
Hironobu Sasano

ABSTRACT

細胞死は正常な細胞でもがん細胞でもみられる現象である。細胞死には，あらかじめプログラムされ細胞の中から積極的に死んでいくアポトーシスや，外的要因によって引き起こされるネクローシスなどがある。がん細胞においては，増殖する過程で血管新生が起こるが，がん細胞に栄養を送る血流が不足すると生存に不利な状態になり，ネクローシスあるいはアポトーシスが起こる。また，多くのがん細胞では細胞周期チェックポイントに異常があり，染色体数の異常を起こすため，増殖能の高い腫瘍ほど細胞死がみられる。

がんは外的な攻撃に対し形を変えて対抗する可塑性(plasticity)を有しており，ゲノムの不安定性ががんの可塑性を規定している。そのため，がんの治療においては細胞表面にあって可塑性に影響しない蛋白をみつけ出すことやがんの周辺環境(niche)を変化させることで，がん細胞自身が増殖能を失うような状況を作り出すことなども検討すべきである。これまでの治療は主にがん細胞のみに目を向けていたものであったが，今後は線維芽細胞などの間質細胞を含めがん細胞の周囲にある組織との相互関係から，免疫系を考慮した治療法に期待が寄せられる。また，がん細胞だけでなく間質細胞などでも発現する微量な分子を検出することで治療による効果を予測あるいはモニタリングする方法も，今後さらに進めていくべきであろう。

なぜ細胞は死ぬのか

戸井　では，「細胞死」というテーマで座談会を始めさせていただきます。細胞死は，がんにおいて最も重要なプロセスであり，治療効果をみるポイントの1つにもなります。また，正常な組織でも細胞死は重要な役割を担っていることが知られています。本日は，分子メカニズムあるいは分子病理学といった観点から細胞死について考えていきたいと思います。まず佐谷先生，細胞はなぜ死ぬのかということについて，どのようにお考えになっていますか。

佐谷　われわれの体の中の細胞数は限られていますので，細胞が生まれてから増殖して分化し，最終的に老化した場合，その細胞は組織から消滅していく必要があります。つまり，ターンオーバーしていくためには，どうしても細胞死は必要なのです。細胞死のこうした現象が観察された当初は，消極的な現象だと考えられていました。それぞれの動物が歳をとって死んでいくように，細胞も歳をとって死んでいくという現象にすぎないと考えられていたわけです。

ところがそのうち，発生の段階で老化もしていないのに細胞が積極的に死んでいき，それによってわれわれの体の形が整えられていくという現象が観察されるようになりました。その最も良い例は手です。手はうちわのような形から発生し，指と指との間の細胞が細胞死を起こして欠落することによって指ができていきます。ですから細胞死とは決して消極的な現象ではなく，実は体の中で積極的かつ生理的に営まれている現象であることがわかってきました。

戸井　その積極的な細胞死の発見が，その後の研究の起点になったわけですね。

佐谷　そうです。細胞死という現象が特定の分子によって行われていることが後に明らかになり，その分子を阻害すれば細胞死が起こらないために発生異常が生じる，あるいはそれが病態につながると考えられるようになりました。細胞死は体にとって必要であると同時に，細胞死が過度に起こることが疾患につながるといった解釈が出てきたわけです。

したがって，細胞がなぜ死ぬのかという質問への回答は非常に難しいと思いますが，生理的な意味では細胞は死ぬことによってわれわれの体全体のバランス，あるいは形というものを作り出しているということになると思います。

戸井　積極的な細胞死はリモデリングにもつながってくるということですか。

佐谷　はい。細胞数が過剰に増えるような現象が起こったときは，それに対してブロックをかけて殺していくという現象が起こります。リモデリングしている過程で細胞を作りすぎてしまった場合，それを積極的に殺していくというプログラムがあるのです。

また，細胞は分裂・複製という過程を繰り返すことによって増殖していきますが，増殖の際にマイナーなミステイクは常に起こります。遺伝子に損傷が起こったり，染色体に傷が入ったりするのです。そして，傷がついた細胞を体に残しておくと，その細胞が変化してがんになることもあります。それを防ぐために，積極的にその細胞を殺していくということが常時行われています。これは一見，病理的な細胞死にみえますが，実は生理的なものであって，いわゆる品質管理を行うために細胞死が積極的に行われていると考えられています。

戸井　では笹野先生，細胞死について，特に病理学の立場からどのようにお考えでしょうか。

笹野　まず考えられることは，増殖する細胞と死ぬ細胞の割合のバランスです。細胞死に変化がなく細胞増殖が増加する，あるいは細胞増殖には変化がなく細胞死が減少すると，過形成になります。長期的にみれば，このように細胞の数を調整していくことで生体は恒常性を維持しています。

また，たとえば日に焼けると皮膚が黒くなることはよく知られています。日光の紫外線に曝露すると細胞のターンオーバーが亢進して，細胞が表皮の上方により速く移動し，死んで剝がれ落ちます。このように，植物でいうと defoliation，つまり葉が落ちるためには，細胞は死ななくてはなりません。この落葉を起こしているのが細胞死なのです。

病理学的には，日光に触れるとそこに含まれている紫外線がDNAを傷害します。そうするとこのDNAの傷を修復しようと細胞がいわゆる修復酵素を出して

戸井　雅和 氏
Masakazu Toi
京都大学大学院医学研究科外科学講座乳腺外科学教授

1982年広島大学医学部卒業。1988年広島大学医学博士号取得。同年，広島大学助手。1990～1992年 Oxford 大学臨床腫瘍学，および分子医学研究所分子血管新生研究室に留学。1992年東京都立駒込病院に赴任。1993～1998年東京大学人類遺伝学非常勤講師。2000年より Harvard 大学，Dana-Farber 癌研究所に留学(UICC Yamagiwa-Yoshida fellow)。2002年より東京都立駒込病院部長。2007年より現職。乳がんの臨床，トランスレーショナルリサーチ，新しい分子標的治療法の開発に携わる。

きます。しかし，どうしてもこのような修復が追いつかず，異常な遺伝子が残ってしまう場合は，その細胞を殺さなければ遺伝子異常を有する細胞ができて皮膚に残ってしまうので，排除しようとします。たとえば，細胞の中に細胞死に向かわせる p53蛋白が増え，細胞を殺す方向に進みます。このように，次世代の細胞を生みだすためや組織の恒常性を保つため，あるいは傷害された遺伝子が次世代の細胞に及ぼす悪影響を除外するために，細胞は死んでいるのではないかと思います。

細胞はどのように死ぬのか

戸井　細胞の死に方にもさまざまな形がありますが，細胞はどのようにして死ぬのかについて，佐谷先生，いかがでしょうか。

佐谷　1つは，従来ネクローシスといわれていたもので，物理的，化学的な傷害によって細胞膜が破れ，細胞の内容物が外に出てしまって死んでいく細胞死です。もう1つは，いわゆるアポトーシスと呼ばれるものです。アポトーシスの定義も最近は非常に難しくなってきていますが，従来の定義でいえば，アポトーシスとはシグナルが働き細胞の中から積極的に死んでいくものであり，細胞膜が保たれた状態で縮んでいき，最終的には細胞の内容物を小さな袋に包み込んだ形で死んでいく細胞死です。

ネクローシスは細胞の内容物あるいはさまざまな酵素が外に出てしまうため，免疫反応や炎症反応を惹起しますが，アポトーシスは細胞内部から死に，それを小さく折りたたんでいくため，生体に対して影響の少ない死に方であると考えることができます。

また，アポトーシスは蛋白質分解酵素であるカスパーゼという分子が活性化されることによって起こると考えられてきましたが，最近ではカスパーゼが活性化しない，カスパーゼ非依存性のアポトーシスもあることがわかり，細胞の死に方にはさまざまな様式があることが明らかになっています。

戸井　ある細胞が死ぬと，免疫反応によってその周囲の細胞まで影響が出ることがあります。免疫反応はどれくらい関わっているのでしょうか。

佐谷　細胞の中の内容物が出ると，おそらくマクロファージなどがそれを貪食することによって，さまざまな免疫反応が励起されると思います。免疫反応の起こり方はどんな細胞が死んでいくかによってさまざまです。また最近，アポトーシスでも死んでいくときに小さく縮んだ細胞は，マクロファージによって処理されていることがわかってきました。アポトーシスのシグナルが活性化すると細胞膜の構造が変わり，自分を食べてほしいという "eat me signal" が表に出てきて，マクロファージがそれを認識し，細胞を食べて処理します。ですから，アポトーシスも最終的には免疫