

## Cancer therapy with local oncolysis and topical cytokine secretion

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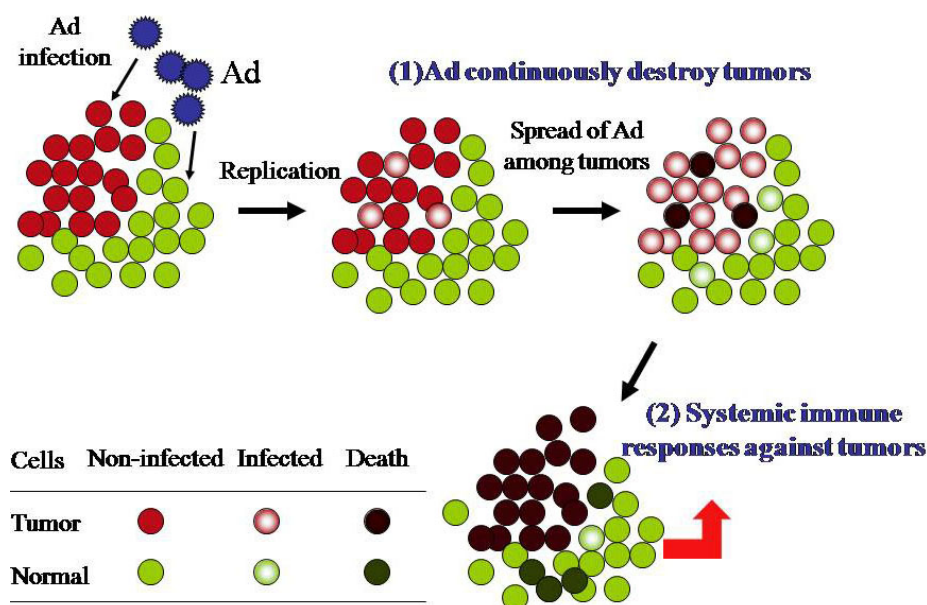
## 1. ABSTRACT

Direct destruction of targeted tumors and subsequent induction of systemic immunity is not pertinent to gene therapy but gene therapy is probably the most suitable therapeutic modality to achieve the local and systemic anti-tumor effects. Current strategies for cancer gene therapy in fact consist of direct inhibition of tumor growth and activation of systemic host defense mechanisms. We have been working on development of oncolytic adenoviruses and cytokine-mediated activation of host immune systems to produce better therapeutic effects. The adenoviruses in which the E1A expression is controlled by an exogenous regulatory region are preferentially cytotoxic to target tumor cells depending on the specificity of the regulatory region and cytokines that differentiate naïve T cells into T helper type 1 cells can amplify immune responses generated. Combination of the two strategies has an advantage. Tumor destruction by oncolytic viruses does not impair immune systems in contrast to chemotherapy and radiotherapy but enable to produce anti-tumor responses against putative tumor antigens that are subsequently released from the destroyed tumor. In this process, dendritic cells play a pivotal role since they act as professional antigen presenting cells and are involved in an initial phase of immune responses, either activation of immunity or induction of immune tolerance. Antigen loading with subsequent appropriate activation of dendritic cells is thereby crucial for activated anti-tumor responses, which possibly eliminate even distant metastatic foci. Combinatory gene therapy with oncolytic viruses and activation of host immune system thereby can evoke immune responses against all the tumor antigens expressed by the process of "antigen-spreading" mechanisms.

## 2. INTRODUCTION

The current gene therapy for cancer contains a number of strategies and could be classified as either direct or indirect actions to tumor cells. The former includes induction of apoptosis by manipulating gene expression relevant to cell survival or cell death, and forced cell death by cytotoxic agents. These methods need high efficacy of gene transfer to target cells and controlled gene expression in the cells. The latter strategy is to regulate tumor growth in the milieu of host responses, which include suppression of angiogenesis and activation of immune systems. Chemotherapy also has similar direct and indirect actions; however, the cytotoxic actions often resulted in immune suppression and are incompatible with immune therapy. Instead, gene therapy is less harmful to immune systems and in particular oncolytic viruses, used for local tumor destruction, rather activates host immunity.

Immune systems, when adequately stimulated and subsequently break immunological tolerance for tumors, can produce tumor regression. A topical treatment, if it achieves cell death of a fraction of the tumors, can trigger host defense mechanisms. The linkage of the two strategies, local tumor destruction followed by activated systemic immunity, hopefully produces synergistically therapeutic effects. We seek for a possible combinatory use of local and systemic treatments, which can be compatible with host defense systems and accordingly produce anti-tumor effects. We speculate that virus-mediated destruction of tumors, even a part of the tumors, will induce the release of putative tumor antigen(s) without suppressing host immunity and that the virus-mediated treatment subsequently activates anti-tumor immune responses (1) (Figure 1).



**Figure 1.** Benefits of oncolytic Ad in cancer gene therapy. Infection of Ad causes destruction of tumors and Ad subsequently released continuously infects the tumors in the vicinity. In the process of tumor destruction, putative tumor antigen(s) are liberated. DCs capture the antigen(s) and generate systemic immunity when they are activated. Even though destruction of tumors is partial, host immunity, if appropriately activated, can eliminate distant metastatic foci. Treatment with oncolytic Ad produces direct cytotoxic effects and indirectly systemic effects through immunity.

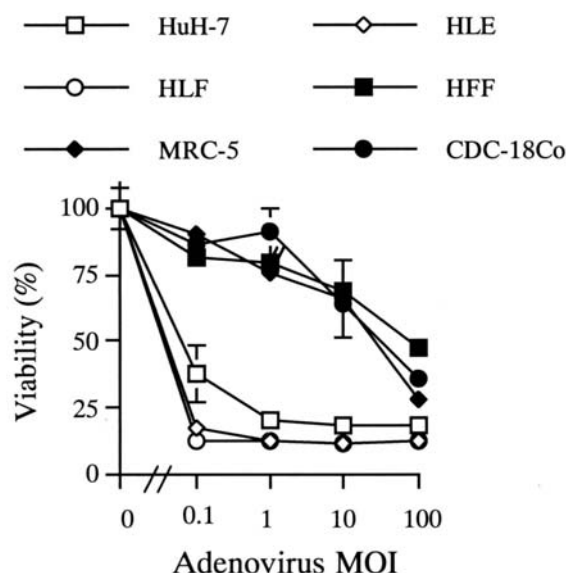
Processing of exogenous antigen(s) by dendritic cells (DCs), one of the major antigen presenting cells, is an initial and crucial phase or induction of systemic immunity. Subsequent activation of DCs increases the antigen expression in the presence of the major histocompatibility complexes (MHC), which determines outcomes of immune responses, generation of immunity or tolerance induction (2). We thereby investigated biological significance of the molecules expressed on immature DCs to seek for augmentation of DCs' functions. We also examined possible application of cytokines to magnify further the immune responses once activated (3). In this article, we discuss the modification of oncolytic adenoviruses (Ad) to improve its infectivity to tumors, Fas/Fas ligand (FasL) interactions on DCs to facilitate antigen uptake and the functions of novel heterodimeric cytokines belonging to the interleukin (IL)-12 family to enhance systemic immunity.

### 3. ANTI-TUMOR EFFECTS PRODUCED BY REPLICATION-COMPETENT AD

There are two major types of recombinant Ad that are capable to replicate and exhibit preferential cytotoxicity in target cells (4-7). One type is to control the Ad *E1A* gene expression with a putative tumor-specific or tissue-specific transcriptional regulatory unit and the other is to engineer Ad to propagate within tumors by deleting a part of the Ad DNA region to which tumor suppressor gene(s) can functionally bind. A well-known example of the deletion-type Ad is ONYX-015, which lacks the E1B-55 kDa-encoding molecule, and the Ad were initially regarded to replicate preferentially in tumors that are

devoid of the P53 functions (8-10). Subsequent studies however showed that Ad replication and the consequent cytotoxicity were not specific to tumor cells or directly related to the status of the *p53* gene (10). Recent reports explained the mechanism of the selective cytotoxic activities to tumors as the enhanced export of viral RNA, which occurred only in tumors (10, 11). Combinatory use of chemotherapeutic agents with ONYX-015 to enhance the anti-tumor effects has been also tested (12, 13). Our recent studies on esophageal carcinoma also confirmed that the ONYX-015-mediated cytotoxic activity did not correlate with the *p53* status and that combinatory treatment with chemotherapeutic agents, although depending on the agents, enhanced the cytotoxicity to esophageal carcinoma tested. The agents however could not produce synergistic anti-tumor effects to tumors if they inhibit the proliferation of Ad. We then examined the possible inhibition by the agents and found that the agents did not always inhibit the Ad proliferation although depending on the administration schedules. These data collectively suggest that combinatory use of oncolytic Ad with anti-cancer agents currently in clinical use will achieve better anti-tumor effects. Although mechanisms of oncolytic Ad-mediated cell death need further analyses, oncolytic Ad can be used for as a local therapy together with chemotherapeutic agents. There seems to be no disadvantage for the combination and both therapies are clinically compatible.

Although a phase III study with ONYX-015 for head and neck cancer (Onyx Pharmaceuticals) was prematurely halted in the States, a Shanghai-based



**Figure 2.** Cytotoxicity of AdMK to hepatocellular carcinoma cells (open symbols) and normal fibroblasts (closed symbols). Cells were infected at various MOI and the viability was examined with the WST assay. Standard error bars are also shown.

company, Shanghai Sunway Biotech, recently acquired the Chinese FDA approval of H101 (Oncorine), which is functionally identical to ONYX-015 (14). Their clinical outcomes obtained with Chinese patients, combination of H101 with cisplatin for head and neck cancer, are reported to be similar to those with Onyx Pharmaceuticals. These encouraging data, although precise comparative analyses are probably required, hopefully boosts clinical application of oncolytic virus therapy not only in China but in other countries. We are currently considering a possible clinical trial with ONYX-015 for esophageal cancer, which is also squamous cell carcinoma of the upper gastrointestinal tract.

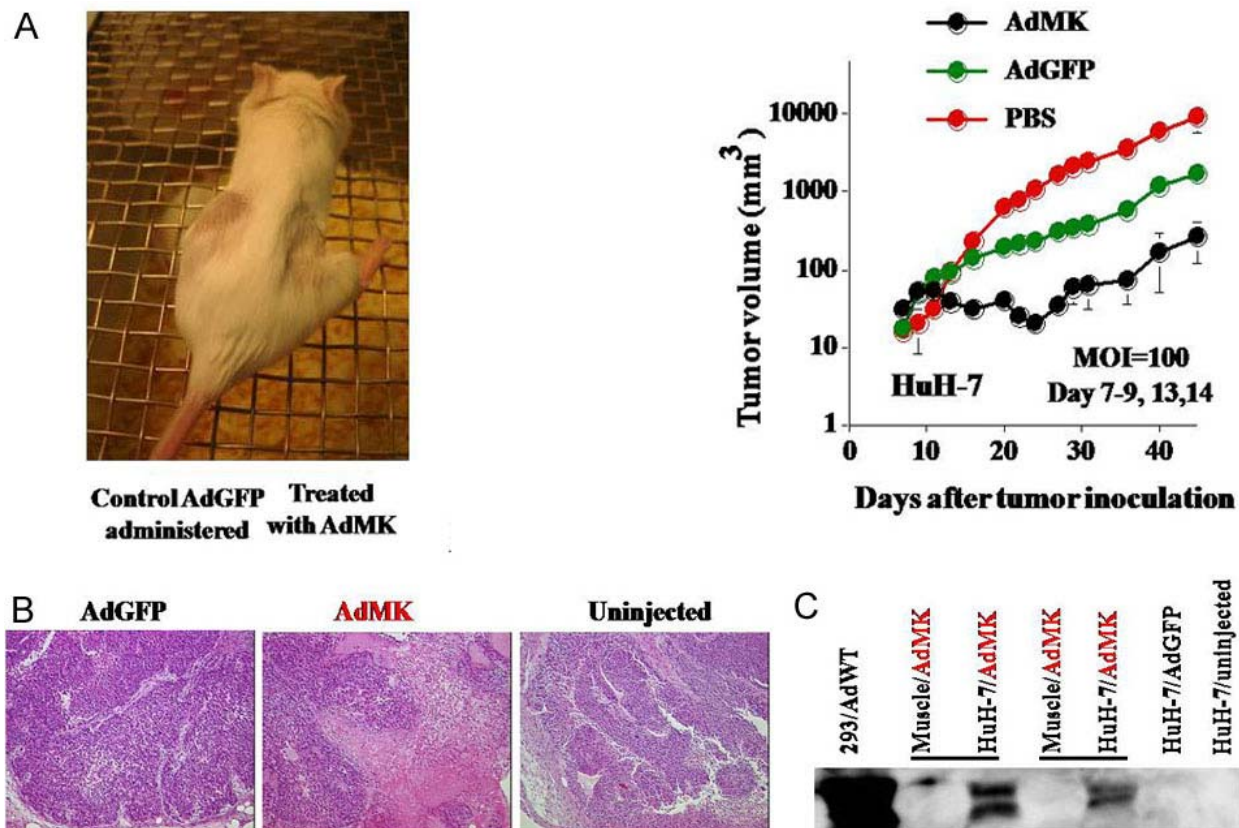
We have been investigating on transcriptional regulatory regions of the genes that are preferentially expressed in tumors. We therefore took the first approach to induce selective destruction of tumors with controlled E1A expression that was driven by a putative tumor promoter. A number of promoters of tumor-related genes such as alpha-fetoprotein and c-erbB-2 were analyzed but the activities were often tissue-specific and may not be applicable to a variety of tumors (15-17). We then examined the regulatory regions of genes that were possibly linked with cell proliferation. In particular we focused on midkine (MK), a heparin-binding protein expressed in embryonic brain, with multiple biological functions (18-21). The expression is down-regulated in adult normal tissues but the expression is elevated in a number of human tumors including gastrointestinal tumors, breast cancer and lung cancer. Our analysis showed that a 600-bp region upstream of the initiation site conferred tumor-specific expression of a linked reporter gene and the MK promoter region could activate exogenous gene(s) preferentially in tumors (22). Tumors but not normal

fibroblasts, both of which were transduced with a suicide gene linked with the MK promoter, became susceptible to a prodrug (22). We also found that a 400-bp region of regulatory region of the *survivin* gene, of which the expression is up-regulated in G2/M phase, activated a reporter gene expression in proliferating cells. The expression is cell cycle-dependent and is enhanced in most of human tumors with unidentified reasons. These transcriptional regulatory regions of the growth-related genes can alter tumors susceptible to cytotoxic agents that are activated by the regions and be applicable to activate the Ad *E1A* gene.

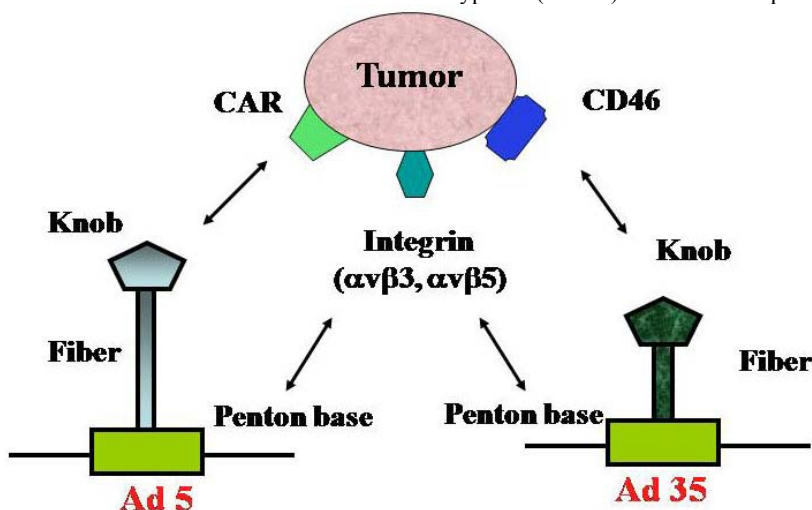
The Ad *E1A* is an immediate early gene product and plays a pivotal role in cell cycle progression and the viral production; consequently, transcription of the *E1A* gene can determine the viral propagation in the infected cells. Regulated expression of the *E1A* gene with a regulatory region can therefore induce virus-induced cell death in the target cells. We constructed Ad type 5 (Ad5) in which the MK promoter activated the *E1A* expression (AdMK) (23). We examined cytotoxicity of AdMK using normal parental fibroblasts and their transformed cells. AdMK induced cell death of the transformed cells at a lower multiplicity of infection (MOI) than that of the normal parental cells. The cytotoxicity of Ad5MK was also greater in a number of tumors than in several kinds of normal fibroblasts (Figure 2). Accordingly, the viral propagation of AdMK in tumor cells was greater than that in normal fibroblasts. These data confirmed the oncolytic activity of AdMK *in vitro*. We intratumorally injected AdMK into established human tumors in nude mice and demonstrated that the oncolytic AdMK produced anti-tumor effects *in vivo* (Figure 3). Administration of AdMK into local tumors is therefore a possible cancer therapy with tumor-specificity. Recently, the *MK* gene is found to be expressed in endothelial cells and consequently AdMK is cytotoxic to tumors also by destroying blood vessels.

#### 4. IMPROVED CYTOTOXIC ACTIVITY OF FIBER-MODIFIED AD

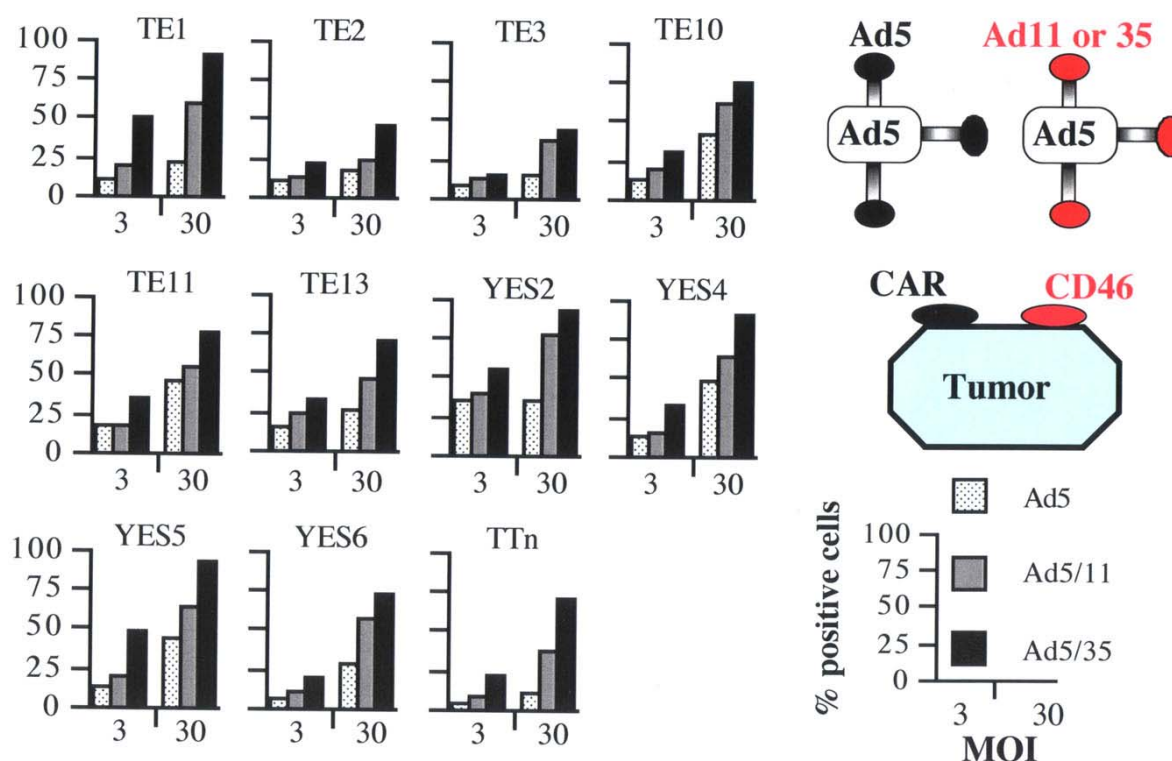
Adverse reactions induced by non-specific Ad integration can be diminished by increased infectivity as well as regulated *E1A* expression. Enhanced infectivity of Ad to tumors decreases the amounts of Ad administered and thereby increases the safety of gene therapy. Attachment of Ad to target cells is primarily dependent on the binding of the Ad fiber-knob portions to the cellular receptors expressed and secondly on the interaction between Ad penton bases and integrins (CD51) on the targets (24) (Figure 4). Since the major cellular receptor of the Ad5 is the coxsackie-adenovirus receptor (CAR), the infectivity of Ad5 is often influenced by the expression level of the CAR on target cells (25). The levels on tumors are however inconsistent irrespective of the tumor types and the expression is sometimes down-regulated; consequently, these tumors are relatively resistant to Ad5-mediated gene transfer. There are two main ways to circumvent the lower transduction efficiency of Ad5 to such tumors. Modification of Ad5 with antibody that inhibits the fiber-knob region/CAR interactions and with



**Figure 3.** (a) Growth of HuH-7 hepatocellular carcinoma developed in SCID mice after the tumors were treated with AdMK, AdGFP (replication-incompetent GFP-expressing Ad) or phosphate buffered-saline (PBS). When the tumors became 4-5 mm in diameter, AdMK, AdGFP or PBS was injected intratumorally on day 7-9, 13 and 14. (b) Histological analysis of the HuH-7 tumors that were injected with AdMK or AdGFP and uninjected HuH-7 tumors (hematoxylin-eosin-stained). (c) Expression of E1A analyzed with Western blot analysis. Two E1A bands migrated about at 48 and 38 kDa. The samples of muscle and tumor were obtained from the same mice. HEK293 cells infected with wild-type Ad (AdWT) were used as a positive control.



**Figure 4.** Receptors for Ad5 and Ad35. The fiber-knob regions of Ad5 and Ad35 bind to CAR and CD46 molecules, respectively. Integrin is a subsidiary receptor binding to the penton base structure.



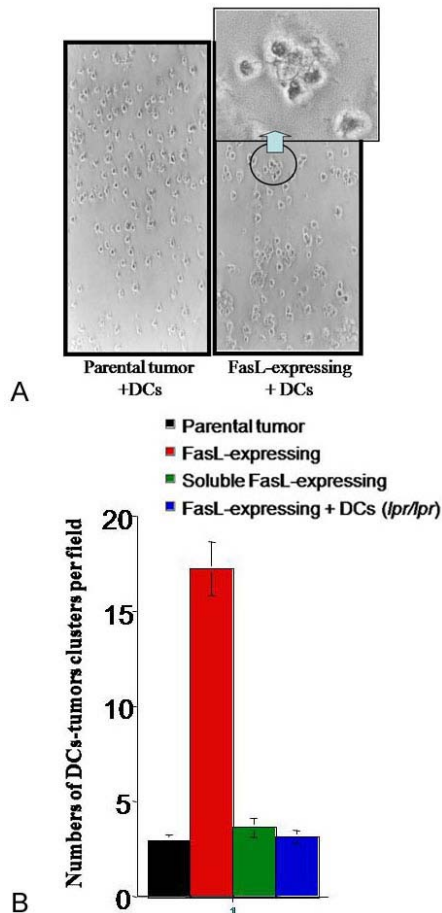
**Figure 5.** Percentages of GFP-positive cells examined with flow cytometry. Eleven kinds of esophageal carcinoma cell lines were infected with Ad type 5 bearing cytomegalovirus promoter-linked GFP gene (Ad5GFP), Ad5GFP in which the fiber-knob portion was replaced with that of type 11 (Ad/F11GFP) or with that of type 35 (Ad5/35GFP). These cells were infected with respective Ad for 30 minutes at a MOI of 3 or 30, were washed to remove Ad and then cultured for 2 days. Cells whose fluorescence was greater than the brightest 5% percent of uninfected cells were judged as positively stained.

insertion of a binding motif to cell surface molecules are possible strategies to generate Ad with retargeting ability to the tumors expressing the surface molecules. The other method is to replace the fiber-knob region with those of other type of Ad, which consequently changes the tropism of Ad infectivity. We tested the efficacy of fiber-knob substitution because the replacement has wider applications.

Among several Ad serotypes, we examined the Ad type 35 (Ad35) as a possible fiber-knob alternative. Ad35, which cause urinary tract infection in human, use CD46 as one of the receptors and the CD46 expression is in general rather up-regulated in human tumors compared with normal tissues (26). We examined the expression level of the CAR, CD51 and CD46 on esophageal and other tumors and found that the CAR expression level was variable among a number of tumor types despite their constant expression of CD51 and CD46 (27). In particular the CD46 expression in the tumors was higher than that in normal fibroblasts. We thereby presume that replacement of the fiber-knob portions of Ad5 with that of Ad35 (Ad5/35) could increase the infectivity to the tumors without changing the well-known properties of Ad5 (Figure 5).

We prepared Ad5 and Ad5/35, both of which harbor cytomegalovirus promoter-linked with green fluorescence protein (GFP), and examined the expression level of GFP in infected tumors with flow cytometry. Although the GFP intensity in each cell line is influenced by a number of factors including the promoter activity in the cells and Ad transportability, the percentage of GFP-positive populations and the mean fluorescence intensity in the same cell line can represent the infectivity of Ad and accordingly the efficacy of Ad-mediated transduction. We showed that HEK293 and human hepatoma HuH-7 cells, which express CAR and CD46 at a high level, became GFP-positive with Ad5 or Ad5/35 vector even at a low MOI. In tumors whose CAR expression was relatively lower, the percentages of GFP-positive cells were greater with Ad5/35 vector than with Ad5 vector when infected at the same MOI (27). According to our analysis on many kinds of human tumors including esophageal carcinoma cells showed better infectivity with Ad 5 than that with Ad5/35 vector (Figure 5). These data suggest that oncolytic Ad5 bearing the Ad35 fiber-knob portions could produce greater anti-tumor effects than conventional Ad5-based oncolytic viruses. We also examined the infectivity of Ad5 bearing the type 11-derived fiber-knob portions (Ad5/11) since the cellular receptor of Ad11 is also CD46 (28). The





**Figure 6.** Cluster formation between FasL-expressing tumor cells and DCs (a) Photograph of the clusters between tumors and DCs. (b) The number clusters found between tumors (Fas-negative parental, FasL-expressing and soluble FasL-expressing) and DCs (purified from syngeneic and Fas-defective *lpr/lpr* mice).

infectivity of Ad5/11 was greater than that of Ad5 in tumors with low CAR expression but was not as great as that of Ad5/35 (Figure 5). The reason of the lower infectivity of Ad5/11 is currently unknown.

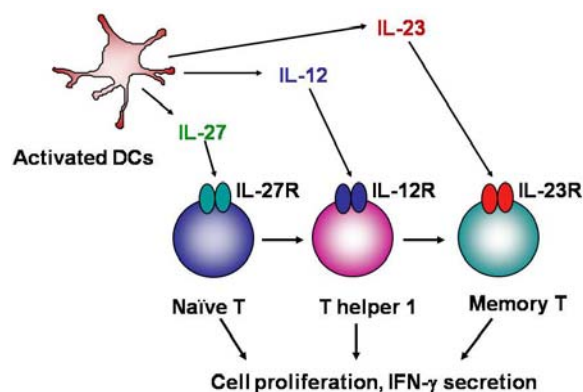
Several data suggested that oncolytic Ad5-mediated anti-tumor activity *in vivo* was not as significant as that *in vitro*. It could be due to anti-Ad immune responses, humoral and/or cellular immunity, and heterogeneous cellular populations of solid tumors. Tumor masses themselves also contain many sorts of normal cells that constitute stroma tissues including fibroblasts and microvessels. These non-tumorous tissues in tumor masses can decrease the cytotoxicity by oncolytic Ad-mediated *in vivo* because they are derived from many cell lineages and devoid of a strong proliferation activity. One of the strategies to circumvent the "barrier-effects" is to develop oncolytic Ad that are also cytotoxic to the stroma. Ad5/35 is a better vector system than Ad5 in this point, increasing the infectivity to stroma cells. We found recently that Ad5/35 had greater transduction capability than Ad5 to

fibroblasts. Since the primary administration route of oncolytic Ad is local and intratumoral, Ad5/35 may not cause strong damages to normal tissues despite their infectivity to normal cells. The cytotoxic activity will be limited to stroma tissues although further investigation is required in the point. Recent studies demonstrated that mesenchymal stem cells (MSC) migrated into tumorous tissues and contributed to formation of stroma components (29). Injection of labeled bone marrow-derived MSC into tumor-bearing mice showed accumulation of the stem cells around tumors. These data thereby suggest that oncolytic Ad, which can propagate in such stroma cells by use of an appropriate promoter, can produce greater anti-tumor effects *in vivo*. Cell growth-related transcriptional activity as shown in MK and survivin promoters could be useful to construct oncolytic Ad since stroma cells proliferate according to the tumor growth. Injection of MSC infected with oncolytic Ad could be one of the therapeutic strategies to overcome the "barrier-effects". The chimeric Ad5/35 vector also has an advantage to efficiently transduce DCs with an exogenous gene in contrast to Ad5 (30). The property is useful for DCs to express exogenous antigens as well as cytokines to facilitate systemic immune responses to putative tumor antigens as described below.

## 5. EFFICIENT ANTIGEN-LOADING IN DCS

Activation of cell-mediated immunity is required to eradicate residual tumors and distant metastatic foci, and induces immunological memory to prevent clinical recurrence. Oncolytic Ad can be an effective local therapy but the Ad alone may not be adequate to eradicate whole tumors as mentioned. We believe that induction of systemic anti-tumor activities is mandatory in particular in clinically advanced cases. Induction of systemic immunity followed by local tumor destruction is an attractive approach to achieve tumor-free outcomes. Activation of DCs is probably a vital step to link tumor destruction and induction of immunity (1, 2). Tumor destruction mediated by oncolytic Ad can give danger signals to DCs; subsequently, activated DCs come to recognize putative multiple tumor antigens shared among the tumors despite of their microheterogeneity. In order to facilitate induction of systemic immunity, we investigated the functional roles of the molecules expressed on DCs because an appropriate antigen presentation process by activated DCs can shift local inflammation to systemic immunity.

Immature DCs express can capture exogenous antigens and come to express the antigens with MHC class II upon their maturation. The typical factors involved in the maturation of DCs are CD40L and proinflammatory cytokines such as TNF- $\alpha$ . None of the molecules involved in the antigen processing is however well described. We found that immature DCs expressed Fas, which plays a major role in an apoptotic process, and also examined the biological significance of the expression on DCs (31). Ligation of Fas molecules of DCs with FasL expressed on tumors did not induce apoptosis of DCs but increased cell-to-cell interactions. Staining of DCs and the tumor cells with specific dyes showed that the ligation facilitate DCs-tumor cells cluster formation (Figure 6a).



**Figure 7.** Secretion of the IL-12 family cytokines, IL-12, IL-23 and IL-27, from activated DCs. Expression of respective receptors on T cells is developmentally regulated. The IL-12 family cytokines coordinately influence T cell differentiation and subsequently activate cell-mediated immunity.

The cluster formation was not observed between DCs and FasL-negative parental tumors or between DCs from Fas-defective *lpr/lpr* mice and FasL-positive tumors (Figure 6b). Furthermore, membrane-bound murine FasL is released into culture supernatants by an enzymatic digestion and the clusters were not formed with tumors expressing such a soluble form of FasL, demonstrating that the clustering is solely dependent on the Fas/FasL interactions.

Anti-tumor effects produced by FasL expression was correlated with the cluster formations. Murine tumor cells expressing FasL were rejected in syngeneic mice and subsequently tumor-specific protective immunity was induced, whereas the anti-tumor effects were not achieved in Fas-defective *lpr/lpr* mice (31, 32). Moreover, tumor cells expressing soluble murine FasL also developed tumors in syngeneic mice (33). The role of Fas/FasL interactions in the antigen presentation was evidenced by DCs that were incubated with FasL-expressing tumors and then injected into syngeneic mice, in which experiment DCs were firstly reacted with FasL-expressing tumors, separated from the tumors and then mixed with tumors. DCs incubated with the FasL-expressing tumors produced anti-tumor effects against the parent tumors but not irrelevant tumors. These data collectively demonstrated that the Fas/FasL interactions facilitated antigen loading in DCs, which subsequently activated T cell-mediated immunity. Supposed that the cluster formation can bridge tumors and DCs, adhesion of tumors and DCs with a "paste-like" interaction may enhance the antigen presentation. It is currently unclear whether the bridging between tumors and DCs is enough to enhance antigen presentation or other biological interactions through the Fas/FasL ligation are additionally required for the antigen acquisition process.

Upon the activation of DCs, they come to secrete a number of cytokines which mediate cell-mediated immunity. We thereby investigated whether FasL

stimulation induced cytokine production in DCs. We examined the expression of cytokine genes in DCs that were incubated with FasL-expressed tumors and found that the FasL-stimulated DCs did not produce any kinds of cytokines tested. In contrast, when DCs were cultured with CD40 ligand (CD40L)-expressed tumors, they secreted a variety of cytokines including IL-12, IL-18 and IFN- $\gamma$  (34). We however did not detect significant numbers of clusters between DCs and CD40L-expressed tumors as found in the incubation with FasL-expressed tumors. Taken together, interaction of Fas/FasL thereby enhances acquisition of exogenous antigens into DCs but does not induce the activation, whereas CD40/CD40L ligation rather induces activation of DCs and subsequently cytokines production. We tentatively presume that the CD40/CD40L pathway itself does not directly contribute to enhanced antigen presentation due to their poor cluster formation activity. Subsequent question is why we observed the anti-tumor activity with DCs stimulated with FasL-expressing tumors, although DCs were not activated. We speculate that injection of DCs itself can give the danger signals to them. Inoculation process would generate proinflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  and these cytokines can contribute to DCs maturation. Likewise, the CD40/CD40L ligation may also enhance antigen processing to some extent and legitimate distinction between the antigen uptake process and the antigen expression in the MHC class II complex could be difficult. We assume that the signal pathways involved in the bipolar functions of DCs could be differentiated by the properties of antigens. For examples, mycobacterial antigens can stimulate DCs through the CCR5-mediated pathway (35).

## 6. AUGMENTATION OF CELL-MEDIATED IMMUNITY BY CYTOKINES RELEASED FROM ACTIVATED DCs

A number of cytokines are secreted from activated DCs and contribute to proliferation and differentiation of T cells. Recent studies showed that novel cytokines belonging to the IL-12 family are produced upon the activation of DCs. The cytokines coordinately induce immune responses since their receptor expressions are sequentially observed in T cells upon their activation and differentiation (Figure 7) (36). The IL-12 family cytokines form a noncovalently linked heterodimer, consisting of p35 and p40 for IL-12, p19 and p40 for IL-23 (37) and p28 and Epstein Barr virus-induced protein 3 for IL-27 (38). Among the IL-12 family cytokines, IL-27 is firstly secreted after the DCs activation and acts on primarily naïve T cells. IL-27 induces the expression of the IL-12 receptors, IL-12R $\beta$ 1 and IL-12R $\beta$ 2, on naïve T cells; thereby, IL-27 actions precede IL-12 functions, which play key roles in the differentiation process of T helper type 1 (Th1) cells (38, 39). We showed that IFN- $\gamma$  production from naïve T cells was enhanced by synergistic actions of IL-27 and IL-12, supporting that both cytokines can shift immune responses toward the cell-mediated immunity. We then examined whether local secretion of IL-27 from tumors could achieve anti-tumor effects. Our study demonstrated that the IL-27 production induced cell-mediated immunity

and that natural killer and cytotoxic T cells were responsible for rejection of IL-27-producing tumors. Recent studies however using mice lacking IL-27 receptors suggest that IL-27 also contribute to the down-regulation of T-cell-mediated immune hyperactivity (40, 41). Although the same gene-targeted mice showed impaired IFN- $\gamma$  production in an early phase of Th1 development after microorganism infection (42), these mice produced excess amounts of inflammatory cytokines and suffered from strong inflammation in a later phase. It is currently uncertain that IL-27 itself is involved in the negative regulation of the cytokine production and we therefore need further investigation on the ambivalent functions of IL-27. The mice defective of the *p28* gene, when available, may explain the dual functions of IL-27-mediated signaling.

IL-23 is also involved in maturation and differentiation of Th1 cells and in particular can facilitate the generation of memory T cells. Others and we demonstrated that IL-23 secretion from tumors induced systemic immunity against the tumors (43-45) and that the anti-tumor activity of IL-23 depended on CD8-positive T cells (43). The memory T cell inducing activity of IL-23 was evidently shown with DNA vaccine, in which administration of IL-23 DNA together with an antigen-encoding DNA maintained the long-term immunity in contrast to other cytokines (46). A different biological activity of IL-23 is recently reported that IL-23 induces IL-17 (47) and that Th-17 in the context of the IL-23/IL-17 axis can be crucial for induction of autoimmune and inflammatory diseases (48). The IL-23 functions in immune reactions are thus complicated as well as IL-27. Although complexities of the cytokine-mediated reactions remains uncharacterized, we hopefully can manage to manipulate anti-tumor responses with the cytokines, understanding the cross-talk among the molecules.

## 7. PROSPECT

The monotherapy with oncolytic Ad will not produce sufficient anti-tumor responses to eradicate tumors. The therapy is essentially local treatment and needs other systemic therapies. Immunology-based therapy and chemotherapy are options to be combined with the virus-mediated therapy. Activation of systemic immunity followed by local tumor destruction is not a novel concept. Low dose of radiation and a minimal dose of an anti-cancer agent will rather enhance immune responses. These phenomena could be due to uptake of released tumor antigen(s) by DCs. Compared with the conventional therapies, tumor destruction with oncolytic Ad does not damage a host immune system but rather increases the activity. The advantage of the virus-mediated therapy however can be a drawback to the virus-mediated tumor destruction. Enhanced immunity is linked with inhibitory actions against multiple administration of the oncolytic Ad. Combinatory chemotherapy can also decrease sufficient proliferation of the viruses, which inhibit subsequent spread of the viruses. These combination modalities therefore have a potential propensity of "double-edged sword". As a next step to achieve better therapeutic effects and to avoid the unnecessary actions to Ad, we need to understand the

generation of immunity against not only tumors but also the viruses and shed light on an appropriate use of oncolytic Ad from the points of topological and chronological administration.

## 8. ACKNOWLEDGEMENTS

This work was supported by grants-in-aid for the COE research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Japan Society for the Promotion of Science (JSPS), the Uehara Memorial Foundation, Kashiwado Memorial Foundation for Medical Research, Chiba Industry Advancement Center (Innovative Technology and Advanced Research in Evolutional Area), Tsuchiya Foundation and the Futaba Electronics Memorial Foundation.

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**Abbreviations:** DCs: dendritic cells; MHC: major histocompatibility complexes; FasL: Fas ligand; Ad: adenoviruses; IL: interleukin; MOI: multiplicity of infection; CAR: coxsackie-adenovirus receptor; GFP: green fluorescence protein; MSC: mesenchymal stem cells; CD40L: CD40 ligand

**Key Words** Adenovirus, E1A, Cytokine, Antigen presentation, Fas ligand, IL-12 family, Review

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