Current Advancement in Radiation Therapy for Uterine Cervical Cancer

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Radiation therapy is one of the effective curative treatments for uterine cervical cancer. However poor clinical results for the advanced stages require further improvement of the treatment. Intensive studies on basic and clinical research have been made to improve local control, primarily important for long term survival in radiation therapy. Regarding current advancement in radiation therapy for uterine cervical cancer, the following three major subjects are pointed out; technological development to improve dose distribution by image guided radiation therapy technology, the concomitant anticancer chemotherapy with combination of radiation therapy, and radiation biological assessment of the radiation resistance of tumors. The biological factors overviewed in this article include hypoxia relating factors of HIF-1α, SOD, cell cycle parameters of pML, proliferation factors of Ki67, EGFR, cerbB2, COX-2, cycle regulation proteins p53, p21, apoptosis regulation proteins Bcl2 and Bax and so on. Especially, the variety of these radiation biological factors is important for the selection of an effective treatment method for each patient to maximize the treatment benefit.

INTRODUCTION

Cervical cancer is a significant cause of death in women worldwide. The combination of external beam radiotherapy (EBRT) and intracavitary brachytherapy (ICBT) is considered the standard treatment for uterine cervical cancer. ICBT has the advantage of delivering a very high dose to the central tumor and a lower dose to the surrounding normal structures, such as the bladder and rectum, resulting in high local control while minimizing normal tissue damage.

In the early stage of the disease, cervical cancer is highly curable by radiotherapy alone. However, the treatment results of locally advanced disease have been poor, with 5-year survival rates of 30–55% for stage IIIB, and 4–20% for stage IVA disease. One of the major causes of death for the locally advanced disease is persistent or recurrent pelvic disease. The analyses of failure patterns following radiotherapy in locally advanced disease showed locoregional recurrence of 50–70% of the patients treated, and this proportion increased with increasing tumor bulk.

Hence further successful treatment strategy should be explored to obtain better outcomes for the locally advanced disease, and three major subjects are depicted: 1) technology development to attain improvement of dose distribution, 2) the use of more attractive anticancer agents than standard chemotherapeutic drugs to enhance tumor response to RT, and 3) advance in radiation biology to determine tumor radiosensitivity before the initiation of RT.

Image guided brachytherapy for improvement of the dose distribution

Although three-dimensional (3D) imaging technique using computed tomography (CT) and 3D dose-volume assessment in dose distribution is usually adopt for EBRT, ICBT is still coordinated by doses to reference points and doses of organs at risk (OARs), that is, the rectum and the bladder, are also assessed not by dose volume but by point dose in the treatment for cervical cancer. To assess the dose to irregular shaped tumors and to optimize the dose based on the dose constrains at every session of ICBT using CT or magnetic resonance imaging (MRI) can give a sufficient irradiation dose to the target with the minimal dose to the OARs at the same time.

Recently, the effectiveness of 3D technique on ICBT for cervical cancer has been proven by several investigators. The first promising clinical result of 3D image-based adaptive ICBT has triggered off its establishment in a large number
Chemoradiotherapy

Chemoradiotherapy (CRT) is considered as standard therapy for locally advanced cervical cancer based on favorable results of phase III clinical trials and a large meta-analysis in 1990s that evaluated the effectiveness of concurrent combination of RT and chemotherapy in comparison with RT alone. These studies demonstrated that CRT had a significant survival advantage of 10–15% at 5 years after treatment compared with RT alone. Nevertheless, loco-regional control and overall survival in patients with stage IIIA–IVA diseases are still unfavorable when compared with those with stage IB–IIB diseases. Additionally, the concurrent CRT could salvage only additional one patient out of the 10 patients by addition of chemoagents and all the patients suffered from acute side effects and some chronic toxicity. One of possible explanations for the unsatisfactory results was to involve non-effective patients to the specific treatment, which resulted in a reduced power of a statistically significant benefit and unnecessary burdens by giving biologically-targeted drugs. These results suggest that more extensive efforts is required to improve CRT and that more precise stratification of the patients strictly effective for chemotherapy is mandatory.

To identify biological markers predictive for effective treatment modalities based on basic research is very attractive to further improve treatment outcomes and or maximize efficiency of CRT for locally advanced cervical cancer.

Biological factors for prediction of treatment outcome

As mentioned above, it is essential to obtain local tumor control to lead successful outcomes after radiation therapy, but it is also well-known that a certain part of tumors dose not respond similarly to the standard RT. Wide heterogeneous treatment response of individual tumors influences significantly on treatment outcomes, even if patients have similar clinical backgrounds, that is, tumor stage, tumor size, histology, and so on. Therefore, if sensitivity to irradiation of individual tumors is predicted before treatment by novel radiological technique, suitable treatment strategy including adequate radiation dose, combination of chemical agents during radiation therapy and other molecular targeting drugs etc. can be delivered.

We had histologically examined tumor response to radiation therapy using cancer specimens according to Ooboshi-Shimosato histological classification by drill biopsy performed at the end of treatment and a month after RT. Visible residual tumor cells were detected in about a half of cancer specimens collected from patients locally controlled. Hence it is too early to decide histological tumor response at the end of RT for cervical cancer. Approximately a month was required to assess histological response because of slow degeneration of tumor cells after RT. In addition, there is a limitation for assessing radiation biologic behavior of the tumor cells with the H&E staining specimen before RT. Hence, other approaches such as immunohistochemical staining methods or DNA analysis are required to analyze molecular characteristics of individual tumors and to apply to clinical strategy for improvement of the outcomes.

The authors have been therefore exploring various biologic factors relating to radiosensitivity and treatment outcomes of the uterine cervical cancers mainly by immunohistochemical analysis. These biologic factors include proteins relating hypoxia, cell proliferation, cell cycle regulation, apoptosis, and oncogenes, in addition to direct oxygen measurement.

The authors review mainly the biologic markers we previously reported.

Growth fraction (GF)

Studies on the growth fraction (GF) of tumors determined immunohistochemically by the Ki-67 antibody (Ki-67 growth fraction) demonstrated that the population of quiescent cells (G0 cells) which are relatively radiation resistant was larger than the cycling cell population in many cancers. Moreover, a wide range of G0 cells population was observed in tumors with same histology. Hence, G0 cell behavior may have an important role in the radiation response of the tumors. The previous studies showed that higher GF tumors were more proliferative and led to poorer survival after surgical treatment than lower GF tumors. However, we proved that the tumors with higher GF were
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not resistant to irradiation but radiation sensitive because local control rates of the tumors with high GF determined by Ki-67 immunohistochemical staining was higher than those with low GF in the cervical cancer patients.36,37 The radiation response may be associated with degree of recruitment phenomenon of G0 cells into the cell cycle during the early period of RT for cervical cancer.33,37

**Mitotic Index of proliferating cell population (pMI)**
Recently, some tumors cause rapid proliferation by stimulation of irradiation during the course of RT and its phenomenon is called “Accelerated proliferation”. The repopulation of tumor cells by “Accelerated proliferation” increases dose required to control all tumor cells and is an important cause of acquiring tumor radioresistance. Hence shortening overall treatment time (OTT) is an important strategy to achieve local control of the tumors which often causes “Accelerated proliferation” during RT.38 A study from the European Cooperative Radiotherapy Group demonstrated in a dramatic way that accelerated treatment using hyperfractionation only for fast-growing tumors with Tpot less than 4 days resulted in substantially better local control than the conventional protocol in head and neck cancer.39 Similarly, previous literatures also revealed the effects of Tpot and OTT on the treatment outcomes of cervical cancer.36,41 Detecting rapidly proliferating tumors before initiation of RT seems very important for delivering a successful treatment to overcome accelerated repopulation of the tumors. However, there are few clinically available markers or indexes to measure proliferative activity or cell cycle speed and we therefore proposed pMI, the mitotic index specific for the proliferating cell population, to assess them in clinical setting.

It is well-known that mitotic index (MI) reflects cell cycle speed in vitro but does not in vivo because of a significant difference in the presence of G0 cells. The authors excluded G0 cells by immunohistochemical staining with Ki-67 antibody and measured pMI over the whole cycling cells, and pMI can overcome large limitation of GF and Tpot for clinical application.36 As a result, the high pMI tumors frequently developed recurrence and yielded significantly poor prognosis in cervical cancer treated with RT alone.36 The authors reproduced similar results even when patients with cervical cancer received heavy ion radiotherapy using carbon ion beams.42 Hence, high pMI, which means faster cell production or repopulation, may be one of the predictive markers of tumor recurrence after RT.

**Hypoxia and Hypoxic markers**
The existence of hypoxic cells is well recognized as one of the major factors affecting radio-resistance which possibly causes local failure after RT.43,44) Urtasun et al. showed that an in vivo solid tumor contained a certain proportion of hypoxic fractions.45) Recently, many investigators have evaluated pO2 in solid tumors directly by using special electrodes and proved the presence of hypoxic tumor cells in human cancers.46,47) Relationships between intratumoral oxygen distribution and pretreatment tumor characteristics, tumor progression, and poor prognosis have been investigated especially in cervical cancer.48,49 Hockel et al. reported that low pretreatment intratumoral pO2 of the tumor tissue was an indicator of malignant progression of the cervical cancer and a strong prognostic factor of poor survival not only in RT but also in surgical treatment.49) As for local control, lower pretreatment intratumoral pO2 was also correlated with worse local control probability.50-52)

Reoxygenation of tumor is one of the important radiation-induced phenomena and rationale for fractionated RT, and it induces effective re-sensitization of cancer cells, previously in anoxic conditions, to irradiation.53 We investigated the intratumoral pO2 status directly measured before and during fractionated RT for cervical cancer.54 In the study, the reoxygenation phenomenon occurred within 1 week after initiation of RT. Additionally, the significantly better local control for oxygenated tumors at 9 Gy was observed as well as oxygenated tumors before RT. These results indicated that effect of reoxygenation induced by irradiation as well as pre-treatment oxygen status played an important role in local control after RT for cervical cancer.

To overcome some drawbacks of direct intratumoral pO2 measurement including poor accuracy because of wide variety of oxygen status in solid tumors, hypoxic markers such as vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) may be alternative methods for detecting tumor hypoxia and also allow an adaptive treatment according to individual biologic tumor data.

HIF-1 is expressed in response to hypoxia in most cells and activates the transcription of a wide range of genes related to oxygen delivery and to metabolic adaptation (glycolytic enzymes, glucose transporters) under hypoxic conditions.55) HIF-1-regulated gene products are likely to play key roles in tumor progression, aggressiveness and may contribute to the increased resistance of hypoxic tumor cells to chemotherapy and RT.56-59) In addition, radiation-induced hypoxia plays an important role in late rectal injury and the inhibition of HIF-1 reduced the radiation induced late injury.60) HIF-1α is the sole subunit which controls HIF-1 activity. We have examined the expression of HIF-1α on the pretreatment biopsy specimens detected by immunohistochemistry for 38 patients with stage IIIB squamous cell carcinoma of the cervix. High expression of HIF-1α was seen in 17 patients (45%), but there was no correlations between HIF-1α and protein expressions of p53, bax, bcl-2 or HPV infection. The significant positive correlation between high HIF-1α expression and recurrence-free survival rate (p = 0.04) or metastasis-free survival (p = 0.03) in our study suggested that HIF-1α is an important prognostic factor, especially for predicting future metastasis after RT for stage IIIB cervical cancer.59)
**p53 and the factors relating apoptosis**

Apoptosis, which means programmed cell death, is an active mode of cell death which occurs in response to DNA damage by ionizing radiation, ultraviolet irradiation and certain chemotherapeutic agents, whereas membrane damage and others are involved in the cause of apoptosis other than DNA damage. A large number of experimental studies have showed that apoptosis induced by irradiation is a determining factor of radiosensitivity. Further, in RT for cervical cancer, a high occurrence of apoptotic cells after a total dose of 9 Gy was significantly associated with better pelvic control. The mechanism of induction of apoptosis by RT in cervical cancer is fully examined by many studies, and it is well-known that p53 plays a key role in radiation-induced apoptosis. p53 is induced through ATM when cells have received DNA damage by irradiation. Induced p53 has many target genes, such as Bax, Bcl-2, RAD51, and p21. Induction of p53 activates or inhibits these downstream genes and also regulates apoptosis through their functions of DNA repair and cell cycle arrest. Thus, many investigators have shown the impacts of p53 status on treatment outcomes in patients with cervical cancer as well as other cancers. Our previous study demonstrated that p53 gene mutation detected by PCR significantly worsened the local control of cervical cancer treated with RT. There was statistically significant differences in the recurrence free survival rates and the local recurrence free survival rates for p53 wild and mutant groups were observed (p = 0.02 and p = 0.03, respectively). Furthermore, the similar results obtained in the tumors with HPV infections indicate that p53 may be a predictive factor in RT for patients with cervical cancer.

We also studied several roles of p53 related proteins in terms of radiation-induced apoptosis in patients with cervical cancer and showed that Bax, a target gene of p53 and a positive regulator of apoptosis by forming heterodimers with Bcl-2, was significantly induced during RT but Bcl-2 expression was not increased. The results reported by Harima et al. using a similar method support our results, and they also showed Bax up-regulation during RT was correlated with good survival (p = 0.04) but Bcl-2 up-regulation caused poor survival (p = 0.002). These results suggested that the functions related to p53-dependent apoptosis may be preserved in cervical cancer though p53 function was inhibited by HPV infection. Moreover, we investigated the expression of p73, a p53 family gene, using tumor specimens obtained before and 1 week after RT. Because the function of p53 in the cervical cancer cells is usually suppressed by HPV infection, p73, which induces apoptosis and cell cycle arrest in a p53-like manner, may play a compensative role in case of lack of the p53 function in radiation-induced apoptosis. A significant correlation between p73 expression after 9 Gy and induction of apoptosis was present in the patients whose p53 expressions were not increases during RT (p53-nonresponding group) (p < 0.001) but not in the p53-responding group (p = 0.940) in our study. These results indicate that evaluation of p53 related protein expressions such as Bax, Bcl-2, and p73 is important for predicting radiosensitivity emerged through at least the induction of p53 dependent apoptosis.

**Molecular targeting agents (growth factors, COX2, and Mn-SOD)**

Molecular targeting agents may be possible to efficiently increase radiosensitivity of cancer cells when given with RT and also eradicate subclinical metastases by themselves. Above all, epidermal growth factor receptor (EGFR) status and its family have attracted attention as a prognostic indicator in cancer, because of its association with specific processes involved in tumor progression and increased expression in a range of carcinomas. A previous study demonstrated that EGFR inhibition with antibody cetuximab (C225) in combination with RT increased tumor control and overall survival in compared with RT alone. The tumor cells with high EGFR expression determined by immunohistochemical staining are theoretically expected to be radioreistance because of activation of survival signaling through PI3K/Akt pathway. However, there were controversial issues whether EGFR expression decreases radiosensitivity clinically. Fuchs et al. recently assessed the prognostic significance of EGFR correlations in cervix SCC and observed a significant association of HER1 overexpression with favorable outcome (p = 0.016), while overexpression of HER2 and HER3 is associated with poor prognosis (p = 0.006 and p = 0.05, respectively), whereas other results sustain that increased staining EGFR was associated with diminished overall survival. The authors demonstrated that the cerbB2 (HER2) overexpression was associated with the poor local control and survival in RT for cervical cancer. In addition, the tumors with c-erbB-2 overexpression had relatively higher pM1 which indicated the tumors with faster cell cycle time and with highly proliferation. The emerging data suggest that EGFR status may be a valuable prognostic and predictive tool in cervix cancer management.

Cyclo-oxygenases (COX1 and 2) catalyze the synthesis of prostaglandins from arachidonic acid. COX2 is inducible protein by inflammation and is augmented in many types of cancer including cervix cancer. Additionally, COX2 plays an important role in regulation of radiation-induced apoptosis. We reported that COX2 inhibited radiation-induced apoptosis during RT for locally advanced cervical cancer and COX2 expression was a good indicator to predict local tumor control. Several studies largely supported these data. Much evidence from various experimental systems suggests that COX2 is important in carcinogenesis and is upregulated in transformed cells and in malignant tissue. Ferrandina et al. demonstrated that positive expression of COX2 is associated with a worse prognosis and response to conventional treatment and that the ratio of COX2 expressed...
cells in adjacent histologically normal epithelium with tumor was an important prognostic factor for treatment outcomes. Combination therapy of RT with use of a selective COX2 inhibitor may yield improved outcomes for patients with COX2 expressing cervical cancer.

The Superoxide dismutase (SOD) participates in the radiosensitivity of the tumors and decreases radiation effects by catalyzing superoxides produced by irradiation. We also investigated the role of Mn-SOD in cervical cancer treatment with immunohistochemical method using anti Mn-SOD antibody and revealed that the Mn-SOD positive rate of the tumor was 48% and the patients with Mn-SOD positive cancers developed more local recurrence and poorer survival after radiation therapy than in the negative group. The Mn-SOD expression is an important factor for intrinsic radioresistance.

Heavy ion radiotherapy

Particle beams including protons and various heavy ions have superior dose distribution encompassing entire tumor and greater potential of improving local control with minimizing normal tissue damage. The particle beams penetrate tissues and give most energy just before edge of penetration to make sharp dose distribution called Bragg’s peak. When applying radiation therapy, Bragg’s peak is spread out in various degrees to make Spread Out-Bragg’s –Peak (SOBP), which can cover tumor bulks and produce great effects on tumors while minimizing normal tissue damage. Moreover, the high linear energy transfer (LET) particles as carbons have various biological advantages in overcoming radiation resistant nature of tumors. Hence, carbon ion beams have both improved dose localization properties and various biological advantages of high LET radiation, including a decreased oxygen enhancement ratio, a diminished capacity to those observed with low LET radiation. The particle beams penetrate tissues and give most energy just before edge of penetration to make sharp dose distribution called Bragg’s peak. When applying radiation therapy, Bragg’s peak is spread out in various degrees to make Spread Out-Bragg’s –Peak (SOBP), which can cover tumor bulks and produce great effects on tumors while minimizing normal tissue damage. Moreover, the high linear energy transfer (LET) particles as carbons have various biological advantages in overcoming radiation resistant nature of tumors.

The reaction of the host

The radiosensitivity is associated with not only the tumor factors but also the host factors, especially, host anti-tumor immunity. It is known that a lymphocyte infiltrating into the tumor influences long term survival. The authors demonstrated that the local control and survival of the patients whose tumors infiltrated with Langerhans cells and the T-cells had significantly better prognosis as well as local tumor control in compared with their control groups. Thus, immunohistochemical findings of the host anti tumor immunity will be other important factors beside radiation biological nature of tumor itself.

The possibility of the clinical application

The variety of these biomedical factors discussed above as the prediction factors of tumor malignancy or radiation sensitivity can apply to clinical practice as selection indicators for individualizations of the treatment. For example, accelerated hyper-fractionation should be applied to rapidly proliferating cervical cancers detected with high pMI as well as high Tpot.

The concurrent chemoradiotherapy is regarded as a mainstay of the treatment for locally advanced cervical cancer in recent years and suggested more precise application of chemotheraphy for stratified patients strictly effective for the drug as discussed previously. For example, because GF is usually a proliferation indicator but is associated with the radiation sensitive cell population and the less amount of the hypoxic cells and HIF-1α is an hypoxic marker, only uterine cervix cancers with low GF and/or higher HIF-1α should be treated with combination of MMC and radiation therapy. The combination of radiation therapy with various inhibitors of SOD, EGFR/erbB2, COX2, and Bcl-2 will be applied for cervical cancers with high expression of those proteins. In addition, because the tumors expressing those proteins tend to highly proliferative, the patients with those tumors are expected significant therapeutic gain by chemoradiotherapy. Thus, identification of biological markers predictive for effective treatment modalities and appropriate selection of the patients by those biomarkers in order to maximize the treatment efficacy play a key role in the improvement of radiation therapy for advanced cervical cancer.
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