

# Molecular predictive factors of outcome of radiotherapy in cervical cancer

## Minireview

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Radical radiotherapy with concurrent cisplatin-based chemotherapy is an established treatment for cervical cancer patients with stage FIGO IIB and higher. The tumor control can be achieved in 40-80% of patients, the treatment is associated with the risk of late postirradiation complications in 10 – 15% of cases. Detection of the factors predictive for tumor control and late morbidity is a possible direction how to individualize radiotherapy dose and technique. The aim of our review is to summarize results of studies inquiring various molecular markers predicting tumor response to radiotherapy and a risk of late complications.

A lot of candidate molecules were evaluated in histochemical studies: membrane receptors (EGFR, HER-2), cell cycle regulators (p53, p21), proliferative markers (Ki-67), hypoxia and angiogenetic factors (HIF, VEGF), HPV status, and others (COX-2), with promising results in some of them (HPV, HIF-1 $\alpha$ , Ku80, ATM polymorphism).

Microarray studies identified decades of genes with different expression in radiosensitive/radioresistant cervical tumors and sets of genes are able to completely separate responding and nonresponding tumors, but these sets differ across studies. Further well designed studies will be necessary to achieve results matured for use in clinical practice.

*Key words: cervical cancer, radiotherapy, molecular predictive factors, EGFR, HER-2, Ki-67, Bcl-2, COX-2, HPV, survivin*

Radical radiotherapy is an established treatment for patients with uterine cervix carcinoma stage FIGO IIB and higher. At present, radical radiotherapy is mostly combined with concurrent cisplatin-based chemotherapy. With this approach the tumor control can be achieved in 40-80% of patients.

Radiotherapy target volume consists of pelvis in all patients; irradiation of para-aortic lymph nodes is indicated in some of them. Combination of external beam radiotherapy with brachytherapy enables the application of high doses of radiotherapy directly into the primary tumor mass. However, this treatment is associated with the risk of late postirradiation morbidity (e.g. radiation proctitis with rectal bleeding; bowel stenosis, fistula, and ulceration; or radiation cystitis). Severe grade III-IV complications can be found in 10-15% of patients. These complications are often associated with better tumor control, presumably due to higher individual

tissue sensitivity to irradiation [1]. Detection of the factors predictive for tumor control and late morbidity is a possible way how to decrease the risk of treatment complications and how to individualize radiotherapy dose and technique to selected patients.

The aim of our review is to summarize the results of studies inquiring various molecular markers predicting tumor response to radiotherapy and the risk of late complications.

### Prediction of tumor response to radiotherapy

**Candidate genes studies.** A lot of molecules involved in the cellular proliferation, cell cycle control, cancerogenesis, DNA reparation, and apoptosis were evaluated in the role of possible predictors of radiotherapy response. Concrete molecules and appropriate results are presented in table 1.

**Table 1. Immunohistochemical studies**

No correlation with chemoradiotherapy result	Controversial correlation with chemoradiotherapy result	Positive correlation with chemoradiotherapy result
Mdm2	p53	HPV
DNK-PK	p21	pAKT
	Ki 67	COX-2
	EGFR	nitric oxide synthase
	Her2	HIF-1 $\alpha$
	Bcl2	HIF-2 $\alpha$
		VEGF
		NF- $\kappa$ B
		Ku80
		EMMPRIN
		survivin

The p53 gene, present on chromosome 17p, acts as a tumor suppressor gene, controlling entry into S phase of the cell cycle. Wild-type p53 activates p21 and induces G1 arrest in the cell cycle. Mutations in this gene inactivate this tumor suppressor activity and are related to the tumor progression and patients prognosis. Oka et al. [2] and Tsuda et al. [3] reported a significant correlation of p53 overexpression and poor outcome of cervical cancer. Conversely, in the studies of Lu et al. [4] and Yamashita et al. [5] p53 overexpression was not correlated with prognosis.

The p21/waf1/cip 1 gene encodes a protein that inhibits a variety of cyclin-dependent kinase complexes and triggers G1 arrest or apoptosis in cells with the wild-type p53 gene. Niibe et al. [6] suggest the possibility that p21 might be a potential suppressor of radiation-induced apoptosis in cervical cancer during radiotherapy. Other studies [5,7] indicated no significant correlation between p21 status and prognosis in cervical cancer treated by radiotherapy.

Ki-67/MIB-1 monoclonal antibody recognizes proliferating cells in the G1, S, G2, and M phases of the cell cycle. The tumors with high Ki-67 expression developed more frequently recurrence and yielded significantly poor prognosis in cervical cancer patients treated with radiotherapy alone in some studies [8]. Other studies failed to show any relationship between the number of positively stained cells and prognosis [9,10,11].

Studies with epidermal growth factor receptor (EGFR) and its family brought conflicting results. HER-2 overexpression was a favorable prognostic factor in the study of Lee et al. [12] and negative prognostic factor in the studies of Fuchs et al. [13], Gaffney et al. [14], and Nakano et al. [15]. Kihana et al. [16] found that increased expression of HER-2 correlates with a poorer prognosis in patients with cervical adenocarcinoma. The study of Yamashita et al. [5] reported a correlation of borderline significance between HER-2 overexpression and unfavorable outcome in patients with cervical squamous cell carcinoma treated with radiotherapy.

Leung et al. [17] found that HER-2 played a more significant role in tumor progression and invasion of cervical carcinoma

than EGFR, but neither HER-2 nor EGFR was found to carry any prognostic significance. Ngan et al. [18] also described the lack of correlation between clinical prognosis and HER-2 expression.

The Bcl-2 gene acts as a regulator of the apoptotic pathway. In the study of Wootipoom et al. [19] Bcl-2 overexpression was associated with poor disease free survival (DFS), but not with overall survival (OS). Also the studies of Pillai et al. [20] and Rajkumar et al. [21] found poorer survival in patients with Bcl-2 expression. Other studies have not found the prognostic significance of Bcl-2 expression in cervical carcinoma [5,22,23]. Harima et al. [24] have not found a correlation between Bcl-2 expression prior radiotherapy and survival, but Bcl-2 expression after administration of 10.8 Gy was the most important predictor of the treatment outcome.

Beskow et al. [25] studied DNA dependent protein kinase (DNA-PK) which is involved in repair of DNA damage produced by ionizing radiation, but no correlation between the radiotherapy outcome and expression of DNA-PK subunits (DNA-PKcs, Ku86, Ku70) was found. The authors observed increased frequency of DNA PKcs, Ku86, and Ku70 positive cells in residual tumors after radiotherapy [26].

The role of human papilloma virus (HPV) as an etiological factor of cervical cancer has been well established. The predictive role of the HPV status of tumors treated by radiotherapy was explored by Harima et al. [27]. The HPV negative patients had significantly poorer prognosis compared with HPV positive patients in the overall survival and the disease free survival.

In the study of Kim et al. [28] the patients with lower HPV viral load had significantly worse DFS than patients with high HPV viral load. Among HPV positive patients worse prognosis for those with HPV 18, 39, and 45 genotypes was reported by Wang et al. [29].

The phosphatidylinositide 3 – kinase (PI3K)/AKT signaling pathway has recently been demonstrated to be a major survival signals in cancer cells. Phosphorylated and activated AKT (pAKT) leads to inhibition of apoptosis. In the report of Kim et al. [30] a significant association was found between the level of pAKT expression and local recurrence rate in cervical cancer patients treated by radiotherapy.

Cyclooxygenase (COX) is a key enzyme that catalyzes the synthesis of prostaglandines from arachidonic acid. Cyclooxygenase 2 (COX-2) plays a pivotal role in regulation of radiation-induced apoptosis. COX-2 overexpression was predictive factor of worse actrial survival and disease free survival rates with a greater incidence of central and lymph node failure, and even of distant metastases in several studies [31,32,33].

Nitric oxide can stimulate COX-2. Overexpression of nitric oxide synthase was associated with decreased survival and a greater propensity to metastasize in cervical cancer patients treated with radiotherapy in the study of Chen et al. [34].

Hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) is a tumor hypoxia marker and is associated with tumor progression and metas-

tases. Increased expression of HIF-1 $\alpha$  correlates with poorer response to radiotherapy, risk of distant metastases, shorter progression free survival and overall survival [35,36,37]. In the study of Kawanaka et al. [38] strong HIF-2 $\alpha$  expression in tumor infiltrative macrophages was associated with increased risk of local recurrence.

Vascular endothelial growth factor (VEGF) has been shown to have a pivotal role in tumor angiogenesis. The largest study evaluating prognostic value of serum VEGF concentrations published by Zusterzeel et al. [39] proved correlation of serum VEGF levels and DFS and OS suggested in previous studies [40,41].

Nuclear factor kappa B (NF- $\kappa$ B) is a transcription factor that is a part of important pathway leading to treatment resistance. Pretreatment expression of NF- $\kappa$ B nuclear staining correlated with increased rates of local-regional failure, distant failure, disease-specific mortality, and OS in the study of Garg et al. [42].

Ku80 is a gene product of XRCC5 and is associated with repair of DNA radiation damage. In the study of Harima et al. [43] Ku80 negative tumors showed significantly better response than Ku80 positive ones with better overall survival in the Ku80 negative patients. Also in the study of Wilson et al. [44] all tumors with a low number of Ku70 or Ku80 positive cells were radiosensitive with higher survival of the patients whose tumors had a low Ku70 or Ku80 expression.

Overexpression of extracellular matrix metalloproteinase inducer (EMMPRIN), a glycoprotein enriched on the surface of many types of tumor cells has been reported to be linked to invasion, metastases, growth and survival of malignant cells. Expression of EMMPRIN was associated with a decrease in the reduction of cervical tumor following brachytherapy and poor survival in the report of Ju XZ et al. [45].

Survivin is a member of the inhibitors of apoptosis. Local control rate of the cytoplasmic survivin-negative tumors was significantly higher than of the positive tumors in the study of Suzuki et al. [46].

Suboptimal results of research for reliable candidate molecules predicting radiosensitivity and radioresistance is easy to understand considering complexity of cell reaction on radiation damage. Increased expression of one gene in the DNA damage response and repair pathway is probably insufficient to account for resistance to radiotherapy. The complexity is well documented by microarray investigations.

**Microarray studies.** The survey of microarray studies testing radiosensitivity/radioresistance of cervical carcinoma is presented in table 2. Radiosensitive and radioresistant tumors differ in tens to hundreds of genes.

In the study of Klopp and al. [47] expression of 58 genes completely separated patients with and without recurrence. The pathways with the most significant changes in gene expression were the death receptor, integrin signaling, apoptosis, and SAPK/JNK signaling pathways.

Kithara et al. [48] identified 62 genes with most pronounced significance for separation between rediosensitive and radi-

**Table 2. Microarray studies**

Author	Number of patients
Klopp AH (47)	12
Kithara O (48)	19
Quing Y (49)	HeLa cervical cancer cell line
Rajkumar T (50)	25
Wong Y (51)	26
Tewari D (52)	8

oresitant tumors. ALDH1, RBP1, MAP3K2, GBL, and RAB5C were the most upregulated genes in radiosensitive and XRCC5 and LDHA in radioresistant tumors. ALDH1 and RBP1 are involved in retinol synthesis and transport. MAP3K2, GBL, and RAB5C belong to MAP kinase signal pathway. XRCC5 and its product Ku80 are involved in double break repair. High expression of LDHA reflects anaerobic metabolism of hypoxic cells, which is related to radioresistance.

Quing et al. [49] studied gene expression on human cervical carcinoma sublines radioresistant to neutron and to X-ray irradiation. In cells resistant to X-ray radiation 38 genes were upregulated a 3 were downregulated by at least two-fold in comparison with radiosensitive cells. The upregulated genes were members of functional groups involved in repair (ATM, RAD 23A, KUB3, MRE11A, RAD51, RAD52, RAD54, XRCC2, XRCC3, etc.), apoptosis, and cycle arrest (MAPK 12, RAD 17, etc.). The three down-regulated genes were CCNH (excision repair), DDIT3 (cell cycle arrest), and GADD45A (apoptosis).

In the report of Rajkumar et al. [50] radiosensitive and radioresistant tumors differed in expression of 112 genes, the difference was at least two-fold in 20 genes and 7 genes with the most significant value for separation between radiosensitive and radioresistant tumors were identified (UBE2C, MMP3, DCUN1D5, SDCCAG8, IGF2BP2, CCL18, FST).

Wong et al. [51] found 300 genes that classified the samples into radiotherapy resistant and radiotherapy sensitive groups representing a wide spectrum of cellular functions including genes with DNA damage response (damage specific DNA-binding protein1), transcription, cell adhesion, membrane and cytoskeletal and signal transduction functions.

In the *in vitro* study of Tewari et al. [52] a set of 54 genes was able to predict tumors response to irradiation. These genes were members of the functional classes for signal transduction, apoptosis, cell cycle regulation, DNA repair, RNA transcription, protein transport, and other.

### Prediction of healthy tissue complications

In the radiotherapy of cervical cancer not only the prediction of tumor response to irradiation is very important, but also the prediction of treatment toxicity depending on radiosensitivity of healthy tissues. In the breast and prostate tumor radiotherapy the ATM mutations were accompanied by higher radiosensitivity and higher risk of postradiation complications

in homozygous carriers [53,54,55,56], although the predictive value of ATM polymorphism for radiation toxicity is not clear yet. Also significance of other candidate genes, e.g. TGF  $\beta$ 1, Nbs 1, Rad 50, MRE, XRCC1, XRCC3, XRCC5 remains controversial [57].

In the cervical and endometrial cancer De Ruyck et al [58] studied the association of polymorphism in XRCC1, XRCC3 and OGG1 repair genes with the development of late radiotherapy reactions and correlation between *in vitro* chromosomal radiosensitivity and clinical radiosensitivity. XRCC3 IVS5-14 polymorphic allele was significantly associated with the risk of developing late radiotherapy reactions. The XRCC1 codon 194 variant showed a significant protective effect. Patients with three or more risk alleles in XRCC1 and XRCC3 had a significantly increased risk of developing normal tissue reactions. The mean number of chromatid breaks per cell was significantly greater in patients with normal tissue toxicity than in patients with no reactions.

The same authors investigated the association between TGF  $\beta$ 1 polymorphisms and the risk of late normal tissue reactions after gynecologic radiotherapy [59]. Homozygous variant TGF  $\beta$ 1 - 1.552delAGG, -509TT, and 10Pro genotypes were associated with severe clinical reactions.

In the study of Ishikawa et al. [60] early adverse gastrointestinal reactions in patients with cervical cancer treated with pelvic radiotherapy were studied in association with 44 functional single nucleotide polymorphisms of 19 candidate genes. Two haplotypes were associated with increased risk of adverse reactions: the first (rs625120C, rs1890371, rs228589A, rs183460G) located in the NPAT-ATM gene, the second (rs2273535A, rs 1047972G) in the AURKA gene, which is involved in cell proliferation and cancerogenesis.

A relatively large proportion of gene expression studies have reported significant findings and several genetic variants have been appointed promising candidates for prediction of radiation sensitivity/resistance of both tumor and normal tissues. Nonetheless, the findings of the studies have often been inconsistent and non-replication of previous results has frequently occurred. This probably reflects an insufficient understanding of the biology underlying complex process of radiosensitivity/radioresistance. Cellular responses to radiation are mediated at the protein level such that translational regulation, post translational modification and degradation of proteins must add additional levels of complexity to the genomic responses. Recent advances in the field of genome-wide association studies [61, 62] and proteomic studies [63, 64] may allow to obtain a comprehensive view of complex biological system underlying cell radiosensitivity/radioresistance.

## Conclusions

Gene analysis is in general able to predict radiosensitivity or radioresistance of the tumor and healthy tissues; in the cervical cancer the most promising candidates are HPV status, pAKT, cyclooxygenase, HIF-1 $\alpha$ , VEGF, NF- $\kappa$ B, Ku 80, EMMPRIN,

and survivin. Better understanding of the biology underlying cell reaction on radiation damage is necessary. Gene sets defined by microarray studies can be used in prediction of tumor response on radiotherapy, but these gene sets vary from study to study. Most studies are underpowered and neglect other variables, as for example tumor dimension, technique of radiotherapy, patients age, etc.

Well designed international studies are necessary to mature genetic research of radiosensitivity and radioresistance to clinical practices.

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