CHAPTER 9

Genetic polymorphisms and tumor response to radiation therapy in oral cancer

Introduction

Radiotherapy has been in use since the 1950s. Radiation therapy is the use of radiation from x-rays, gamma rays, electrons and other sources to destroy cancer cells. Radiation in high doses destroys cells in the area being treated by damaging the DNA in their genes, making it impossible for them to grow and divide. During radiation therapy, both cancer cells (which are growing in an uncontrolled way) and healthy cells are affected, but most healthy cells can repair themselves afterwards.

Side effects of radiation therapy

The side effects of radiotherapy depend on the treatment dose and the part of the body that is treated. The most common side effects are tiredness, skin reactions (such as a rash or redness, permanent pigmentation, and scarring) in the treated area, and loss of appetite. Radiation therapy can cause inflammation of tissues and organs in and around the body site radiated. This can cause symptoms that depend on what organs are affected and to what degree. For example, radiation can inflame skin to cause a burn or permanent pigmentation. It can also irritate the colon and cause diarrhea. Radiation therapy can also cause a decrease in the number of white blood cells, cells that help protect the body against infection.

Role of biomarkers in assessing tumor response

Oral cancer and head and neck cancer are suitable for evaluating radio therapeutic effects because of easy staging and follow-up of treatment. Most of these cancers respond well to radiation treatment. However, inherent radioresistance is a common phenomenon and usually at least 2 yr of follow-up is needed before any conclusions of the potential cure can be made (302). RT is laborious and exhausting both for the patient and the treatment unit and thus early recognition of responders and nonresponders would be useful. Further benefit would be gained from a method identifying those patients, who could be effectively cured by radical RT.

Radiotherapy is still a major treatment modality for oral cancer treatment. Tumor response to radiation therapy paradoxically depends on the extent of DNA damage induced and its repair. We hypothesized that polymorphic cancer patients might have reduced tendency to develop recurrence due to a deficient repair system. Therefore, in addition to the role of SNPs as cancer susceptibility markers, we also looked into their possible role as biomarkers of radiation treatment (RT) response.

As a concluding part of the study, we looked into the association of DNA repair gene polymorphisms with response of individual tumors to radiation treatment. The oral cancer patients included in the study were followed up for 3 years and tumor and treatment response were recorded. To the best of our knowledge this is a first report which has linked repair gene polymorphisms with treatment efficacy.

Aim

To correlate the presence of the genetic polymorphisms, extent of *invitro* DNA damage with the treatment outcome of oral cancer.

Materials and methods

Study subjects

This study was designed with the same study group used for Micronucleus assay. A total of 100 patients were followed up for a period of three years. Only those patients who completed radiotherapy were included in the study. “Status at last follow up” after 3 years were completed was used as the endpoint. According to the response of tumor, patients were divided into, complete response (CR) and partial response (PR). Complete response (CR) was defined as a complete regression of all visible/palpable tumors and radiographic disease. The patient response to treatment at the end of the follow up period was classified as “No evidence of disease” (NED) and “Patients with event” (event = recurrence, relapse or death). Clinical pathological data were derived from patient records and pathology reports.

Data Analysis

The 2x2 contingency cross-tabulation tables provide the distribution of cases and controls by genotype status for each set of genes. Normal genotype was considered as the referent group.

Number and percentages of cases and controls were tabulated in the table. Odds ratio was calculated to quantify the measure of association with corresponding 95% confidence interval.

PROC FREQ was used to obtain the results to meet the objectives. The CMH option provided the adjusted odds ratio for 2x2 tables. PROC FREQ was further applied to compute the odds ratio estimate using the Mantel Haenszel and logit methods.

The proc freq procedure presents both the Mantel Haenszel and logit estimate for the odds ratio. When any one of the cells in the contingency table was found to have zero counts, then value 0.5 was required to be added to each cell of the contingency tables and thus logit estimate of the common odds ratio was reported. In cases where cell counts were non-zeros the Mantel Haenszel estimate was presented.

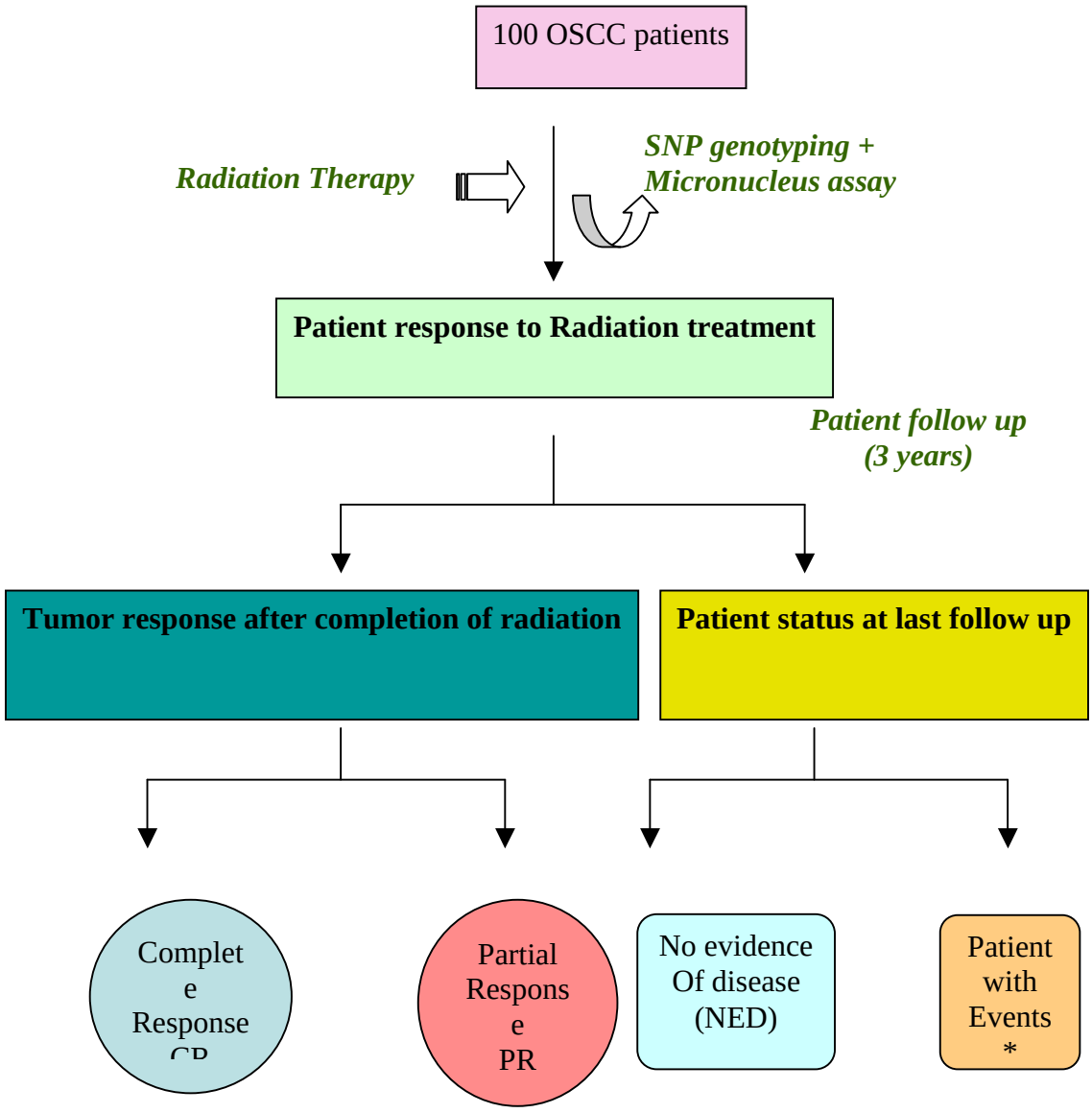
The chi-square or Fisher's exact test was used to measure the extent to which the observed data differed from those expected if the two odds of exposure are equal. Fisher's exact test was performed when the expected frequencies was less than 5 in any cell.

For the confounding factor gender (strata level at female and male), stratum specific estimates and their confidence interval were estimated. The summarization of overall results of the study such that the confounding effect of exposure was also calculated. The CMH option produced the Cochran-Mantel-Haenszel statistics (general association statistic). For this stratified

2×2 table after adjusting for gender, estimates of the common odds ratio and the Breslow-Day test for homogeneity of the odds ratios were also computed.

Fig 9.1

SNPs and treatment response to radiation therapy of oral cancer



Events*= Recurrence, relapse, death

Result

Tumor Response to Radiation treatment

All 100 oral cancer patients underwent radical radiotherapy. This is the same cohort which was also analyzed for Micronucleus assay. Therefore presence of MN and SNPs in DNA repair genes was used as the biomarkers for assessing treatment response. Tumor response to radiation therapy was classified as complete response [CR] or partial response [PR] (Table 9.1a and b). Micronucleus did not seem to have any statistically significant association with response of tumor to RT (p value=0.51). However, tumors of patients with SNPs in their DNA repair genes, XRCC1 (194,399 codon), XPD and ERCC1, responded better to radiation treatment than those with the normal genotype, in the case of XRCC 194 codon [OR =2.56, 95%CI=1.06-6.22], XRCC 399 codon [OR=3.64, 95%CI=1.55-8.53] XPD 751 codon [OR=5.37, 95%CI=2.24-12.81] and ERCC1 [OR=22.8,95%CI=7.5-69.4)

Table 9.1a
Tumor Response to Oral Cancer Treatment and XRCC1, XRCC3 Status In Oral Cancer

GENOTYPE	CR	PR	OR [A] 95%CI)	P VALUE
*XRCC*1 (194 codon) *Arg/Arg* *Arg/Trp+Trp/Trp*	13 48	16 23	2.56 (1.06-6.22)	0.04
*XRCC*1 (399 codon) *Arg/Arg* *Arg/Gln+Gln/Gln*	16 45	22 17	3.64 (1.55-8.53)	0.003
XRCC3 (241 codon) *Thr/Thr* *Thr/Met+Met/Met*	46 15	32 7	1.49 (0.54-4.06)	0.47

Table 9.1b

Tumor Response to Oral Cancer Treatment and NER Pathway Genes Status in Oral Cancer

GENOTYPE	CR	PR	OR [A] 95%CI)	P VALUE
XPD *Lys/Lys* *Lys/Gln+Gln/Gln*	18 43	27 12	5.37 (2.24-12.89)	0.0001
ERCC1 *C/C* *C/T+T/T*	14 47	34 5	22.8 (7.5-69.4)	0.0001
ERCC4 *Arg/Arg* *Arg/Gln+Gln/Gln*	47 14	33 6	1.63 (0.57-4.70)	0.44

Radiation treatment response to oral cancer

The patients who completed the radiation treatment were followed up for a period of three years. They were categorized into patients with “no evidence of disease” [NED] and “patients with event” ie. patients with recurrence, alive with disease or dead with disease after three years of follow up. There were no casualties reported. 71 patients reported as NED after 3 years of follow up while 29 patients were found to be alive with disease owing to local recurrence or due to partial response to radiotherapy. Interestingly, when this was correlated with presence of polymorphisms in DNA repair genes it was seen that polymorphic patients responded better to radiation treatment than those with the wild genotype [Table 9.2]. However, there was only a modest increase in risk associated with oral cancer for all the genes, even though the p values were not statistically significant. The presence of MN was also assessed in terms of treatment response to radiation therapy and it was seen that subjects with normal MN count had more number of events, whereas patients with abnormal MN responded better to treatment [Fig 9.2]. Both presence of MN and polymorphic genotype group were clubbed together to see if there was any association with risk of oral cancer. The p value suggests that there is association between the classified category and status for XPD (p=0.0361) and XRCC399 (p=0.0010) only. All other genotypes did not exhibit any association between higher MN+ polymorphic genotype and treatment response (Table 9.3)

Table 9.2

Association between genetic polymorphisms and treatment response to radiation therapy after 3 years of follow up

GENOTYPE	NED	Patients with event	OR [A] 95%CI)	P VALUE
*XRCC*1 (194 codon) *Arg/Arg* *Arg/Trp+Trp/Trp*	20 51	9 20	1.14 (0.44-2.94)	0.800
*XRCC*1 (399 codon) *Arg/Arg* *Arg/Gln+Gln/Gln*	24 47	14 15	1.82 (0.75-4.40)	0.18
XRCC3 (241 codon) *Thr/Thr* *Thr/Met+Met/Met*	55 16	23 6	1.11 (0.38-3.20)	1.00
XPD(751 codon) *Lys/Lys* *Lys/Gln+Gln/Gln*	32 39	13 16	1.00 (0.41-2.36)	1.00
ERCC1 *C/C* *C/T+T/T*	35 36	13 16	0.83 (0.35-1.98)	0.82
ERCC4 *Arg/Arg* *Arg/Gln+Gln/Gln*	56 15	24 5	1.28 (0.42-3.93)	0.78

Fig 9.2

Association between presence of MN and treatment response to radiation therapy after 3 years of follow up

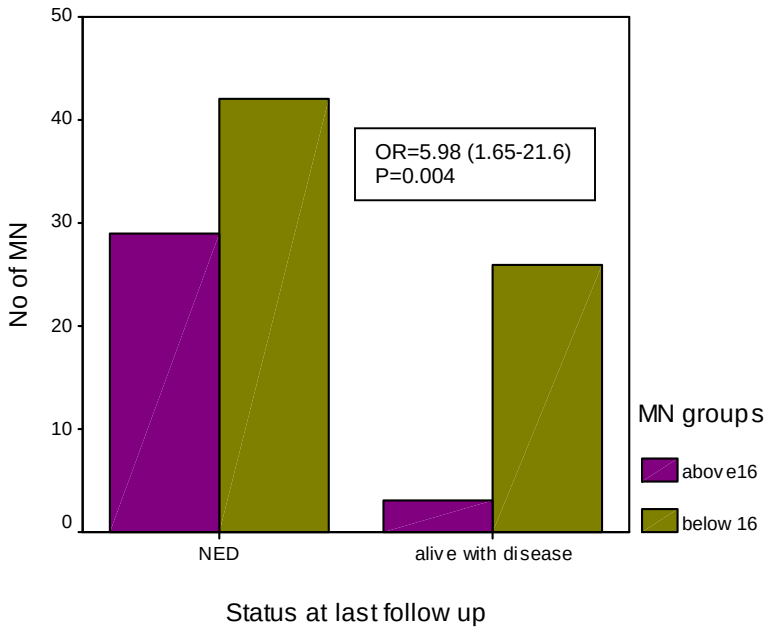


Table 9.3

Risk association among cases in MN and Polymorphic genotype with respect to status

Genotype	Category	Alive with disease	No evidence of disease	Odds Ratio	CI	p-value*
ERCC1	Above 16 MN & polymorphic	1 (1.00)	12 (12.00)	0.14	(0.02, 1.26)	0.0704
	Less than 16 MN & wild	11 (11.00)	19 (19.00)			
ERCC4	Above 16 MN & polymorphic	0 (0.00)	4 (4.00)	0.17	(0.01, 3.28)	0.2855
	Less than 16 MN & wild	21 (21.00)	32 (32.00)			
XPD	Above 16 MN & polymorphic	1 (1.00)	17 (17.00)	0.11	(0.01, 0.96)	0.0361
	Less than 16 MN & wild	11 (11.00)	21 (21.00)			
XRCC194	Above 16 MN & polymorphic	1 (1.00)	11 (11.00)	0.15	(0.02, 1.32)	0.0726
	Less than 16 MN & wild	11 (11.00)	18 (18.00)			
XRCC280	Above 16 MN & polymorphic	0 (0.00)	5 (5.00)	0.14	(0.01, 2.58)	0.1465
	Less than 16 MN & wild	20 (20.00)	30 (30.00)			
XRCC399	Above 16 MN & polymorphic	0 (0.00)	20 (20.00)	0.03	(0.00, 0.64)	0.0010
	Less than 16 MN & wild	11 (11.00)	16 (16.00)			
XRCC3	Above 16 MN & polymorphic	0 (0.00)	5 (5.00)	0.14	(0.01, 2.75)	0.1506
	Less than 16 MN & wild	20 (20.00)	32 (32.00)			

Association of other prognostic factors with MN and polymorphism

Prognostic factors of oral cancer like tumor size (T1-T4), nodal status (N0,N1,N2,N3), site of cancer (buccal mucosa, tongue, floor of mouth, bot, lower alveolus, palate, retromolar trigone, upper alveolus and tonsil) and histology (well differentiated, moderately differentiated, poorly differentiated and nos) were statistically analyzed for association with presence of MN and SNP.

Among cases, the subjects having higher MN (above 16) and carrying abnormal genotype is compared with the subjects having less MN (below 16) and carrying normal genotype, with respect to each of the prognostic factors.

No association was observed between the higher MN+ polymorphic genotype group and tumor stage, site of cancer or histology. However, the p values for nodal status of the genes compared with the presence of MN and SNP group combined showed that except for the XPD gene there exists an association between the two groups (table 9.4).

Table 9.4

Risk association among cases in different categories with respect to node

Genotype	Category	N=0	N=1	N=2	N=3	p-value*
ERCC1	Above 16 MN & polymorphic	5 (5.00)	1 (1.00)	7 (7.00)	0	0.0003
	Less than 16 MN & wild	17 (17.00)	12 (12.00)	1 (1.00)	0	
ERCC4	Above 16 MN & polymorphic	0 (0.00)	0 (0.00)	4 (4.00)	0	0.0008
	Less than 16 MN & wild	26 (26.00)	20 (20.00)	7 (7.00)	0	
XPD	Above 16 MN & polymorphic	8 (8.00)	4 (4.00)	4 (4.00)	2 (2.00)	0.0490
	Less than 16 MN & wild	15 (15.00)	15 (15.00)	2 (2.00)	0 (0.00)	
XR194	Above 16 MN & polymorphic	5 (5.00)	0 (0.00)	7 (7.00)	0	0.0001
	Less than 16 MN & wild	16 (16.00)	12 (12.00)	1 (1.00)	0	
XR280	Above 16 MN & polymorphic	0 (0.00)	0 (0.00)	5 (5.00)	0	0.0002
	Less than 16 MN & wild	25 (25.00)	18 (18.00)	7 (7.00)	0	
XR399	Above 16 MN & polymorphic	10 (10.00)	3 (3.00)	5 (5.00)	2 (2.00)	0.0390
	Less than 16 MN & wild	18 (18.00)	8 (8.00)	1 (1.00)	0 (0.00)	
XRCC3	Above 16 MN & polymorphic	0 (0.00)	0 (0.00)	5 (5.00)	0	0.0002
	Less than 16 MN & wild	27 (27.00)	18 (18.00)	7 (7.00)	0	

Discussion

Radiotherapy (RT) has been used to treat cancers for a century. However, radioresistance remains a major problem in the clinic. It remains difficult to predict the outcome of radiotherapy of cancer despite major progress in diagnosis, treatment planning, and therapeutic modalities. Tumor size, morphology, histology, and other presently used clinical methods for assessing tumor response are relatively nonspecific and imprecise. RT is laborious and exhausting both for the patient and the treatment unit and thus early recognition of responders and nonresponders would be useful. In view of cancer heterogeneity and the availability of an increasing number of therapy options, identification of biomarkers that can predict tumor response to a given therapy is crucial in streamlining treatment and sparing patients from receiving often toxic and expensive therapies that are not likely to be effective.

Most studies pertaining to treatment response to radiation therapy have dealt with either the adverse effects of radiation on patients (303-305) or a gene polymorphism - radiation dosage effect on lymphocytes or gene products as predictive markers (306,307). To the best of our knowledge this is the first study which suggests a role for gene polymorphism in influencing treatment protocols. All the patients were subjected to RT in the study. Interestingly, patients with polymorphic variants in their genome responded better to RT as was evident from their complete response (CR) and no evidence of disease

(NED) after a follow up period of three years. On the other hand a majority of patients with a wild genotype showed only partial response to RT and number of events like recurrence, after the follow up period was high. Most of the subjects who were categorized in the “normal MN” group were carrying the wild genotype and this same group returned to the clinic with various cases of partial response and recurrence. This further emphasizes our hypothesis that genetic susceptibility plays a significant role in DNA damage and development of cancer. Unfortunately, there are limitations of further discussing and analyzing these results due to lack of literature of similar studies.

Nevertheless, these paradoxical results opens up a whole new arena of personalized medicine, wherein genetic susceptibility can be used as a diagnostic tool in the outpatient clinic in deciding treatment options with an expected favorable response from the patient. This however is a preliminary study, and needs to be further validated in larger sample sizes including subjects from different demographic, racial and cultural backgrounds.