Akt1 rs2498801 Is Related to Survival in Head and Neck Squamous Cell Cancer Treated with Radiotherapy

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Abstract. *Background: Radiotherapy (RT) with or without chemotherapy (CT) plays an important role as exclusive treatment in patients with head and neck squamous cell cancer (HNSCC). Unfortunately, in some cases, benefit for patients is not recorded and only treatment-related complications are registered. Materials and Methods: Data relating to Akt1 single nucleotide polymorphism (SNP) and response to treatment of 46 patients treated with exclusive RT or RT-CT for HNSCC were evaluated. Results: For heterozygous patients median overall survival was 28.5 months, while for the wild-type group median overall survival was 10.9 (p=0.019). Three-year survival was 85% for mutated Akt1 homozygosis and 40% for patients with a heterozygous status (p=0.019, hazard ratio (HR)=7.960). Conclusion: SNP of rs2498804 can recognize patients resistant to RT-CT. Further studies are needed to confirm our data and to investigate the role of Akt SNPs in HNSCC patients.*

Squamous cell carcinoma of the head and neck (SCCHN) accounts for over 6% of all malignant cases and radical radiotherapy (RT), either alone or in combination with chemotherapy (CT), plays an important role to improve local control and organ preservation (1). Although the introduction of latest advances in RT and the use of concomitant new drugs against molecular targets (2), not all patients (patients) benefit from standard therapies and in some cases treatment-related complications represent the cause of concern. Currently, only clinical factors, such tumor size, nodal involvement and histological characteristics, have been validated as prognostic factors in HNSCC. However, in patients suitable for exclusive RT or RT-CT, the identification of biomarkers could help to stratify patients in different classes over the possibility to obtain a clinical response or to develop serious side effects.

AKT is a serine/threonine-specific protein kinase, which, once activated by phosphorylation, drives the signal between cellular membrane and the nucleus through the phosphatidylinositol 3-kinase (PI3K)-related signal. Phosphorylated Akt (p-AKT) plays a role in many cellular processes, such as increased resistance to ionizing radiations, survival, proliferation, migration and differentiation (3-6).

Analysis of single nucleotide polymorphisms (SNPs) of AKT1, an isoform of Akt, can be helpful in predicting response to treatments and toxicity in patients with solid tumors (7), therefore the aim of the present study is to evaluate the role of rs2498804 *AKT1* polymorphism in clinical outcomes in patients with SCCHN treated with RT or RT-CT at the University Hospital of Pisa.

Materials and Methods

A total of 46 patients (37 men and 19 women), treated between 2005 and 2009, were evaluated. All patients had a histological diagnosis of squamous cell carcinoma of the head and neck region, assessable disease according to RECIST criteria (8), ECOG performance status 0-2, adequate bone marrow function. RT has been delivered as 3D-RT or intensity modulated RT (IMRT). Some patients were treated with simultaneous integrated boost (SIB). When planned, concurrent CT provided for the combination of cisplatin (100 mg/m^2 q21 or 40 mg/m^2 weekly) or weekly cetuximab 400 mg the first dose and 250 mg the following. Some patients with locally advanced disease were treated with induction CT according to TPF based scheme (Docetaxel, cisplatin and 5-fluorouracil) (9). A total dose of 66-72 Gy was delivered to gross tumor and 50-60 Gy to node; both using standard fractionation. The follow-up examinations with radiological and endoscopic

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exams were planned according to the guidelines of the National Comprehensive Cancer Network (NCCN) 2014.

Genomic DNA was extracted from routine blood samples. DNA was isolated through the use of QIAmp DNA mini kit (Qiagen) with Taqman probe assays by real time polymerase chain reaction (PCR) and the ABI PRISM 7900HT Sequence Detection System software version 2.0 (Applied Biosystems, address). The SNP rs2498804 of *Akt1* was used to divide this cohort into three groups: homozygous AA (wild type), heterozygous AC, homozygous mutant CC.

Data relating to genotype were compared with the response to treatment evaluated according to RECIST criteria and the reported toxicity according to RTOG criteria (10). The different genotypes were correlated with the clinical history of patients in terms of treatment response, toxicity and time to progression. Time to progression was calculated from the date of histological diagnosis until radiological or clinical evidence of disease progression. Overall survival was calculated from the data of histological diagnosis until death. Evaluations of time to progression and survival were performed by the Kaplan-Meier method and differences between groups were analyzed with the log rank (Mantel-Cox). The SPSS program (IBM SPSS Statistics, address) was used for the statistical analysis. For the study of qualitative variables, the X^2 Test of Pearson has been used, while for the parametric variables the analysis of variance (ANOVA).

Results

The median survival was 28.4 months (range=4.1-72). In patients with local failure, the average time to progression was 25 months. Oncology treatments were delivered in the following manner: exclusive RT 12 patients, RT-CT 28 patients, RT + cetuximab 6 patients, induction CT (TPF schedule) 15 patients.

Response to treatment, observed at the first evaluation after the end of RT according to RECIST criteria (based on CT with contrast medium and clinical examination), had a statistically significant correlation with overall survival (p=0.005).

Based on different genotypes, patients were stratified as follow: 17 (37%) mutated homozygous CC, 6 (13%) wild type AA, 23 (50%) mutated heterozygous AC. The analysis of *Akt1* polymorphism showed a statistically significant difference in overall survival (Figure 1) and it was not related to the stage and nodal status. For heterozygous patients, the median overall survival was 28.5 months, while for wild type group patients the median overall survival was 10.9 (p=0.019). Pts with the homozygous mutation did not reach the median time of overall survival during the study period follow-up. After three years, by the end of RT, 85% of patients with mutated *Akt1* homozygosis and 40% of patients with heterozygous (p=0.019, HR 7.960) were alive.

No statistically significant differences were observed for toxicity recorded in patients with different genotype.

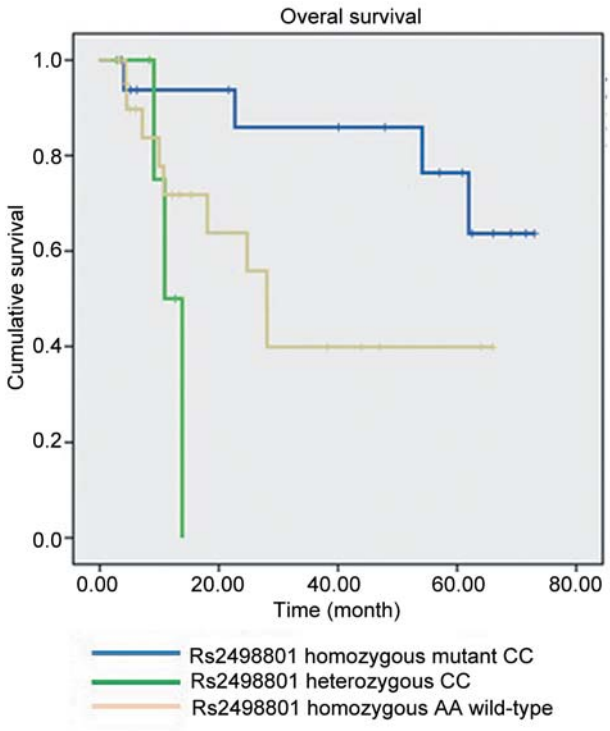

Figure 1. *Overall survival according to the Akt1 rs2498801 genotypes.*

Conclusion

Several papers that appeared in recent years have investigated the role of Akt in patients affected by solid tumors treated with RT or RT-CT (5, 6). In 2002, Gupta and coworkers assessed the role of p-Akt in a series of 38 patients treated with RT-CT for HNSCC. PI3K signaling was evaluated by staining for p-AkT (11). The two-year local control was 100% for patients staining 0-1 + for p-Akt as compared with 70.6% for patients staining 2-3 + (p=0.04). Similar results were found in a study performed at the M.D. Anderson Cancer Center and published in 2005. The expression of p-AKT was detected in 24 patients with tongue cancer. It has been observed that in relapsed patients (15 of 17, 88%) or in patients who died from cancer (10 of 12, 83%) p-AKT was highly expressed and disease free-survival was significantly shorter if AKT was overexpressed (p<0.0001). These results were independent of stage and nodal status (12). Our findings may suggest a role of rs 2498804 to recognize patients with HNSCC resistant to RT or RT-CT. However, the number of analyzed cases in this work is too small to assign a definitive importance to this *AKT* polymorphism in radio-resistance. Even if backed-up by a statistically significant p-value, our results have to be confirmed in a randomized prospective study. Future studies with larger series of patients could even help to predict the onset of important side effects before their clinical appearance and allow the beginning of specific supportive care in order to reduce the risk of high grade toxicity.

Competing Interests

All the Authors declare that in the past five years they have not received reimbursements, fees, funding or salary from an organization that may, in any way, gain or lose financially from the publication of this manuscript. They do not hold any stocks or shares in an organization that may, in any way, gain or lose financially from the publication of this manuscript, either now or in the future. All the authors are not currently applying for any patents relating to the content of the manuscript, have not received reimbursements, fees, funding or salary from an organization that holds or has applied for patents relating to the content of the manuscript.

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