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Hypoxia strongly upregulate the expression of EGFRvIII in glioma cells

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Introduction: Lots of scientific literatures suggest frequent existence and clinical importance of an extra cellular deletion variant of EGFR (EGFRvIII) in a variety of human tumor. The recent data also show using hypoxia inducible factor1 alpha as indicator of tumor responses to EGFR-targeted therapy in preclinical studies and in the clinical setting is expected. Previous work has identified the detection of EGFRvIII in vivo and not in the corresponding cells under in vitro conditions. Then we have therefore hypothesized that its expression might be regulated by the microenvironment.

Materials and Methods: The human glioma cell line (U373 MG) was used and genetically modified to stably express EGFRvIII or a control vector. Cells were grown in a hypoxia chamber under acute anoxic conditions following a prolonged time course of in total 24 hours. Every 6 hours, EGFR and EGFRvIII protein and mRNA expression levels were monitored on Western blots and qRT-PCR, respectively. Phospho- immunoblots quantified the phosphorylation levels of EGFR and its downstream effectors Akt and Erk. Finally, clonogenic survival assays were performed at different time points daily starting after 24 hours anoxia to a maximum of 4 days. Survival fractions were calculated after correction for plating efficiency under normoxic conditions.

Results: The protein but not the mRNA expression levels of EGFRvIII significantly increased under hypoxic conditions compared to EGFR by at least 4 fold. Interestingly, basal phosphorylation levels of EGFRvIII behave differently according to different phosphorylated sites. In line with controls, p-ERK levels continuously decreased during the different time points of hypoxia treatment. In contrast, however, p- Akt levels increased, suggesting a survival benefit for cells with an induced EGFRvIII expression under hypoxia.

Conclusion: This work indicates that EGFRvIII is an important modulator of radiation responses in vivo. In addition, the results suggest an important role for hypoxia in the regulation of EGFRvIII expression in vivo. If this proves to be correct, it would dramatically improve our understanding of the importance of EGFR in radiation responses, since hypoxia is known to negatively influence radiosensitivity in tumor cells.

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Radiosensitization and cell cycle phases distribution induced by paclitaxel in human fibrosarcoma HT1080 cell line in vitro

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BACKGROUND: Recent studies suggest that taxanes cuold be useful in sarcoma treatment. As taxanes are well known radiosensitizers, this study evaluates in vitro radiosensitization by paclitaxel-cremophor in human fibrosarcoma cell line HT1080.

MATERIALS AND METHODS: HT1080 cells were grown in 10% FBS supplemented DMEM. After plating, paclitaxel-cremophor at different concentrations was added to treated wells of a 24-wells plate. After 24 hours, cell cultures were irradiated at 0, 2, 4 and 6Gy and left to grow for 10 days. Citotoxicity was evaluated by the crystal violet method. Distribution in cell cycle and apoptosis was evaluated by DNA stain with propidium iodide and flow citometry analisys.

RESULTS: HT1080 cells were sensitive to paclitaxel with an IC50 range of 4 to 10nM. Dose enhancement ratio (DER) at 0,25 survival level was 1,38; 1,91 and 4,1 for 1, 5 and 7nM drug concentrations, respectively. A marked arrest in G2-M cell cycle phase was observed in treated cells (24% vs. 90,84% for control and treated cells after 12 hours). A moderate amount of apoptosis (23,7%) was observed after 36 hours exposure.

CONCLUSIONS: Paclitaxel radiosensitizes human fibrosarcoma HT1080 cells in vitro by a G2-M cell cycle arrest.

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A clinical study for the adjuvant brachytherapy in the treatment of soft tissue sarcomas

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Background: Soft tissue sarcomas (STSs) bear a risk for local recurrences, especially in high-grade sarcomas. Brachytherapy (BRT) has been used as a tool to obtain better local control in the treatment of STSs. We examined the treatment outcome for STSs using adjuvant interstitial BRT in our single institution retrospectively.

Materials and methods: Twenty-five patients were treated from1994 to 2007 with adjuvant BRT (16 male, 9 female; median age, 51 years). Median follow-up was 37 months (5-146 months). Fifteen patients were treated as primary disease and ten as recurrent. Eleven patients had tumors in lower extremities, 8 patients in upper extremities, and 6 patients in trunk. Histologically, 19 patients suffered high-grade sarcomas, and 6 patients had low-grade sarcomas. Fifteen patients were operated with musculocutaneous flap reconstructions including 11 free flaps after excisions of the tumors. Eight patients were treated with various types of neo-adjuvant chemotherapies. Four patients were pre-treated with neo-adjuvant radiotherapy before the operations. We placed applicators for BRTs in the tumor bed with the cooperation of radiotherapists. The dose of BRT was 6 Gy per fraction, and the frequency was two times a day. The total dose of BRTs ranged from 30 to 42 Gy.

The overall survival rate was 76.3% and 55.5% at 3 and 5 years. Local relapses were observed in 5 patients. The overall local control rate was 87.5% and 75.3% at 1 and 5 year. Wide surgical margins were achieved in 6 patients, marginal margins in 11 patients, intralesional margins in 8 patients. Eight patients experienced complications after BRTs, including infection of surgical site, seroma, delayed wound healing, hematoma, bleeding from pedicle vessels of free musculocutaneus flap and fracture of radiated site. We performed additional surgeries for two cases for the complication.

Conclusions: In our series, we have performed limb sparing surgery in the relatively challenging cases. However, the local control rate after BRTs was almost similar to the previous reports. All of the musculocutaneous flaps were successfully adopted after BRTs, except for one case, which suffered rupture of sutured pedicle vessel. This ruptured case might be associated with fibrosis of surrounding tissues after the BRT, and suggested the need to consider the location of applicators for BRTs.

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Neoadjuvant chemotherapy followed by concurrent chemo-radiation therapy in patients with locally advanced nasopharyngeal carcinoma preliminary results of 21 patients

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Purpose: To evaluate the feasibility and toxicity of neoadjuvant Cisplatinum and Doxorubicin Chemotherapy (CT) followed by a concurrent Cisplatinum Chemo-Radiation Therapy (CRT)

In patients with locally advanced Nasopharyngeal Carcinoma (NPC).

Methods and Materials: Twenty one patients with locally advanced NPC, and without distant metastasis, were treated at the Benbadis University Hospital of Constantine, between January 2006 and June 2007, by neoadjuvant Cisplatinum and Doxorubicin CT followed by Cisplatinum CRT. The median age of patients was 39.8 years range (16-55 years), the sex-ratio was 2.0 (14 male and 7 female). 17 pts (81.0%) had stage III and IV according to the 1997 International Union Against Cancer/American Joint Committee on Cancer classification system. 13 pts (61.9%) had cervical lymph node involvement (N2/N3). Histopathologically, all patients had undifferentiated NPC type. Patients underwent three cycles of induction neoadjuvant CT with Cisplatinum 100 mg/m2 and Doxorubicin 60 mg/m2 on days 1, 21 and 42, followed by a locoregional hyper-fractionated radiotherapy (1.6 Gy twice a day with an interval of 6 hours), with 4 cycles of concurrent weekly Cisplatinum 40mg/m2 on days 1, 8, 29, and 36 of radiotherapy. The total dose to the primary tumor and involved nodes was 70.4 Gy and to the supraclavicular nodes was 45 Gy.

Results: 19 pts completed the scheduled treatment and 2 pts had voluntary stopped the treatment. The response to treatment was evaluated 2 months after the completion of therapy. Objective response (OR) rate was 90.5% (19pts), with 52.4% (11pts) of complete response (CR) and 38.1% (8pts) of partial response (PR). At a median follow-up of 13 months (5-18), 5 pts (23.8%) had failed treatment: one patient (with T4 primary) had a locally relapse after 12 months and 4 pts (all with N3 nodes) developed distant metastases. The median time for first relapse was 11 months (8-16). All patients completed the four concurrent cycles of Cisplatinum. However, 3 of them required the delay of the cycles due to toxicity. Acute Grade 3 and 4 reactions were observed during CRT: leukopenia 42.8% and 4.7%, infection in 14.3% and 0%, mucositis in 66.7% and 4.7%, and skin reaction in 47.6% and 4.7%, respectively. Weight loss was observed in all patients with a median loss of 6.8 kg (4-10 kg) after the completion of the treatment.

Conclusions: Preliminary results show that neoadjuvant CT followed by concurrent CRT is a safe and effective regimen of treatment for locally