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Post-radiation reaction includes induction of fluoropyrimidine metabolizing enzymes – a concept supporting enhancement of capecitabine anabolism regardless of timing administration

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Background: Chemoradiotherapy employing capecitabine became common though not standard treatment approach for rectal cancer as well as for other gastrointestinal tumors. The efficacy seems at least comparable to chemoradiotherapy with continuous infusion of 5-FU. However the plasma levels of main capecitabine anabolites (5-FU, F-dUMP) basically do not simulate a continuous infusion of 5-FU. Presumably the induction of fluoropyrimidine anabolizing enzymes by radiation is responsible for enhanced capecitabine anabolism to active forms. This phenomenon is more decisive for the efficacy of chemoradiation with capecitabine than is the administration schedule. In a previous series of experiments we have proved the enhanced levels of (TP), thymidine kinase (TK), thymidine synthetase (TS) and dihydropyrimidine dehydrogenase (DPD) 24 hours and later after single radiation (200 cGy) in HeLa cells. The experiments were continued to define the development of cellular levels of TP, TK, TS, DPD 8–120 hours after radiation.

Material and Methods: HeLa cells were irradiated by a dose of 200 cGy followed by an array of assessments TP, TK, TS and DPD. A Western blot analysis was performed using specific commercially available antibodies. The time intervals between radiation and onset of increased enzyme concentration were established.

Results: The protein levels of TP, TS and DPD increase from 24 hrs. after the radiation. The increase was up to 5–6 fold and lasted for more than 96 hrs. after the radiation in the first series of experiments. In the following series the increase is much less apparent, not more than 2 fold, however lasts beyond 120 hours. A TP/DPD ratio may be roughly established, in a range of relative values 2–3 in the initial series of experiment and decreased to relative values 1–2 in the following series. Thus the domination of anabolism is much less apparent.

Conclusion: The protein assessments confirm a stable enhancement of fluoropyrimidine anabolism to active forms. The enhancement period far exceeds the intervals between single fractions of radiation and between single doses of capecitabine. The TP/DPD ratio confirms slight excess of anabolism over catabolism. The enhanced anabolism of capecitabine is presumably more important factor than any possible timing of administration related to radiation fractions. Favorable efficacy of capecitabine for chemoradiation does not consist in any simulation of continuous 5-FU infusion

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Viral markers as prognostic factors of cervical lesions progression

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The etiological role of high-risk HPV in preinvasive and invasive cervical cancer has been demonstrated by epidemiological and molecular studies. Taking into account this association, by using viral load and E6/E7 viral mRNA as clinical markers for progression, we try to identify subjects at risk for the development of cervical lesions. Case patients with ASCUS and LGSIL were selected from a cohort of 200 women enrolled in a study on HPV prevalence in different areas in Romania. Subjects were examined at 6 months interval and their cytological and colposcopy data and virological tests (HPV DNA type and load) were monitoring. HPV typing, viral mRNA levels and viral load were determined in cervical-brush specimens at base line and in the samples obtained at 6 month interval.

HPV genotypes were determined by a hybridization assay (Innolipa HPV) and viral load was quantified according to number of copies/ng of β globin. 43 patients (24–48 years) presenting specific viral types (16, 18 and 45 in single or mixed infections) were subjected to viral load testing and viral mRNA detection. As negative controls, cervical specimens from 8 patients without HPV infection and normal cytology were used.

From the 16 patients with ASCUS cytology, 4 subjects presented significant viral loads in both samples; only in one case, the viral load was higher in the second sample. 27 LGSIL subjects presented higher viral load than ASCUS patients (especially in patients with HPV 16 and 16 and 18 mixed infections).

E6/E7 mRNA levels were higher in 69.76% cases which presented infection with HPV16 type (alone or coinfections). From this, only 3 cases were

patients with ASCUS (significant or moderate viral load). In 2 of the 7 cases presenting LGSIL and HPV 18 infections, the viral load and viral mRNA were higher. Both cases came from a Romanian area where this HPV type is found with a higher incidence. Our results indicated that viral persistence, an important factor in cervical lesion evolution, is associated with higher viral loads and mRNA levels but the patients monitoring must be extended for a longer period of time and perhaps for other markers.

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Measurement of DPD and TS transcripts aimed to predict clinical benefit from fluoropyrimidines: confirmation of the trend in Russian colorectal cancer series and caution on the gene-referee.

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Background: Measurement of intratumoral expression of dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) may have some value in predicting the response to fluoropyrimidine-containing therapy.

Materials and Methods: We attempted to validate this association in a series of Russian metastatic colorectal cancer cases. While replicating already published protocols, we unexpectedly found that the use of commonly utilized gene-referees, GAPDH and b-actin, may lead to artifacts due to pseudogene-driven amplification from the genomic DNA template. We have developed a real-time PCR protocol, which amplifies short PCR fragments thus allowing an efficient analysis of archival formalin-fixed paraffin-embedded tumor samples, and relies on SDHA as a gene-referee therefore avoiding an amplification from genomic DNA.

Results: Low content of DPD transcripts was observed in 13/20 (65%) patients with the disease control (tumor response or disease stabilization) as compared to only 3/9 (33%) subjects with progressive disease ($p = 0.11$). Despite the low number of patients, this association reached the level of statistical significance when similar analysis was done for TS expression (11/20 (55%) versus 1/9 (11%); $p = 0.03$).

Conclusions: Thus our data confirm that low DPD and TS expressors have higher chances for success of fluoropyrimidine-containing regimens.

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Small interfering RNA administration against alpha-fetoprotein inhibits proliferation and anti-apoptotic properties of hepatocellular carcinoma cell lines

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Background: Hepatocellular carcinoma (HCC) is the 5th most commonest malignant tumors worldwide. Its incidence is the highest in Southeast Asia and Africa, but the number of reported cases of HCC has been steadily increasing in the United States and Europe because of the spread of hepatitis C virus infection. Current options for the treatment of this cancer consist of include surgical resection, orthotopic liver transplantation, transcatheter arterial embolization, chemotherapy, and percutaneous ablation therapy. Although early diagnosis and treatment improve survival, HCC is rarely cured after these therapies, because multicentric tumor development or intrahepatic metastasis results in frequent recurrence. Therefore, there is a continuing need for new therapeutic strategies are continuously required to impact improve outcome. On the other hand AFP is one of the most important markers in the diagnosis of primary HCC and many other. More than a few Some authors reported AFP as an independent predictor of poor prognosis for HCC. Furthermore, other organ cancers expressing AFP showed aggressive characteristics. It has been demonstrated that AFP ishas been demonstrated to be involved in pleiotropic activities affecting the processes of cell differentiation, cell growth, apoptosis, cytokine production, immunosuppressive activity, and tumorigenesis. These results have an important implication strongly imply that AFP may function as an HCC growth stimulator; thus, the suppression of AFP gene expression and its biological activities may become an attractive strategy for HCC.

Materials and Methods: We analyzed the correlation between serum AFP levels and clinicopathological findings in 37 HCC patients who received curative operation about correlation of serum AFP level and