Expression of a specific Thymidylate synthase polimorfic allele in metastatic colorectal patients is regulated by Myeloid Zinc Finger 1.

A. Calascibetta[1], A. Martorana[1,] M. Labbozzetta[1] and R. Sanguedolce[1]

1 Dept of Sciences for Health Promotion and Mother and Child Care, G. D'Alessandro, Pharmacology Unit, University of Palermo

Background: Thymidylate Synthase (TS) is the target enzyme for fluoropyrimidine anticancer drugs. Its expression is regulated by the number of functional upstream stimulatory factor (USF) E box consensus elements present on its 5' untranslated region. To date are known different polymorphisms, the first one consisting of 2 or 3 repeat of a 28 bp sequence, a further single nucleotide polymorphism (SNP) consisting in a G>C substitution within the second repeat of 3R (3RG>3RC) and recently it has been identified an additional SNP a G>C substitution at the 12th nucleotide in the first repeat of the 2R allele (2RG>2RC). These polymorphisms can influence TS expression, in particular 3R/3R genotype and the presence of 3RG alleles are associated to an increased transcriptional activity and to higher TS levels. The sequence of promoter region of colorectal cancer (CRC) samples was subjected to an in silico analysis (http://www.cbrc.jp/research/db/TFSEARCH.html) to search for all potential transcription factors binding this region. We found that Myeloid zinc finger 1(MZF-1) binds the analyzed consensus. By the literature it is known that this factor induces invasion and in vivo metastasis in CRC, so we investigated a possible correlation between TS and MZF-1 expression in the same pathological samples. **Materials and Methods**: we analyzed the distribution of these polimorphisms in a group of 68 healthy Caucasian subjects, in the normal tissue, in primary tumour and in liver metastasis of 13 CRC patients. Tandem repeat length and the presence of SNP was determined by direct sequencing of genomic DNA. TS and MZF 1 expression were analyzed by immunohistochemistry. **Results**: In healthy population the allele frequency was respectively 2RG(35%) 3RG (44%) 3RC (21%), in colorectal patients while both primary that normal and metastatic samples showed the same genotype: 2RG/3RG. TS and MZF-1 expression were related and gradually increased from normal tissue (negative) to the primary tumour (focally positive) in the metastases (overexpressing). **Conclusions:** These unexpected results lead to the hypothesis of a genetic selection towards a more aggressive disease and enough suggest that regardless of genotype other factors are involved in regulation of TS expression as MZF 1, therefore the only genetic marker is not a valid predictor of eventual fluoropyrimidine response.

References

1)M V. Mandola, J. Stoehlmacher, S. Muller-Weeks et al. A novel single nucleotide polymorphism within the 5'tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 Binding and alters trascrptional activity. Cancer Research(2003); 2898-2904.

2) L F Lincz, F E Scorgie, M B Garg, S P Ackland et al. Identification of a novel single nucleotide polymorphism in the first tandem repeat sequence of the thymidylate synthase 2R allele. Int J Cancer (2007) 120, 1930-1934.

3) M. Morganti, M. Ciantelli, B Giglioni et al. Relationship between promoter polymorphisms in the thymidylate synthase gene and mRNA levels in colorectal cancers. Eur J Cancer (2005) 41, 2176-2183

4)G Mudduluru, P Vajkoczy, H Allgayer Myeloid Zinc Finger 1 induces migration, invasion, and in vivo metastasis through axl gene expression in solid cancer.