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Repair of the mutagenic DNA lesion O⁶-CMG in human colon epithelial cells

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Colorectal cancer (CRC) is the third most common kind of cancer worldwide. Several environmental risk factors are known to contribute to the development of this disease. For example, the consumption of (processed) red meat has long been acknowledged as being involved in the pathogenesis of CRC, a fact recently confirmed by the International Agency for Research on Cancer (IARC). Consumption of red meat entails the endogenous formation of nitroso compounds (NOC´s), which are known to induce a variety of DNA lesions. Most of them are repaired by different repair mechanisms such as the removal of the damaged base by O⁶-MeG-methyltransferase (MGMT) or the base excision repair pathway. Even though MGMT repairs O⁶-methylguanine adducts, there is an ongoing discussion whether this enzyme is actually capable of repairing O⁶-carboxymethylguanine (O⁶-CMG), a specific DNA adduct induced by NOC´s. O⁶-CMG is known to be present in colon cancer cells after red meat consumption and is a highly mutagenic DNA lesion. It was therefore of great interest to clarify whether the O⁶-CMG adduct is a substrate for the MGMT and consequently to shed some light on the role of this enzyme in colorectal cancer protection. For this purpose, non-malignantly transformed human colon epithelial cells (HCEC) were transfected with shRNA in order to suppress MGMT expression, which was confirmed by an activity and expression analysis. Possible geno- and phenotypic changes of the transfected cell line were investigated by testing for anchorage-independent growth in the growth in low attachment (GILA) and soft agar assay and growth inhibition by testing the saturation density in cell culture. As no differences between the transfected and the parental cell line were observed, both cell lines were then treated with azaserine, a substance known to induce O⁶-CMG adducts. Potential DNA damage induced by this compound was detected via single gel electrophoresis, and it could be shown that MGMT-deficient HCEC cells were highly sensitive to the azaserine treatment. To further understand the role of MGMT in the repair of DNA lesions, a quantification of DNA adducts using LC-MS/MS techniques will be performed in order to investigate whether more O⁶-CMG adducts can be detected in MGMT-deficient cells and whether MGMT is responsible for the repair of the O⁶-CMG lesions.

Biography

J Döhring studied Cell Biology at the University of Osnabrück, Germany. Afterwards she attended the Master's program "Animal Biology and Biomedical Sciences" at the University of Veterinary Medicine Hannover, Germany. Since 2014, she is working as Doctoral student in the Institute of Food Toxicology and Analytical Chemistry at the University of Veterinary Medicine in Hannover. She investigates the influence of red meat consumption during colon cancer formation.

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