ABSTRACT

Fanconi Anemia (FA) is a heterogeneous syndrome genetic and phenotypic and sensitizes to alkylating agents that generate DNA crosslinks (ICLs). FA patients have a high risk of a squamous cell cancer of the head and neck (HNSCC). Interestingly, patients with FA and HNSCC does not differentiate between genetic changes as compared to sporadic HNSCC.

The aim of the dissertation was to identify the genes from the FA/BRCA pathway deregulated in LSCCs, to characterize them at molecular levels and to investigate the DNA repair capacity after cisplatin treatment in regard to selected FA protein presence.

The 21 LSCC cell lines, 101 DNAs from primary LSCC tumors, 55 DNAs from healthy margins and 35 RNA primary LSCCs and controls were used in this study.

In order to meet the aims of this project the palette of methods was used, pyrosequencing, DNA copy number microarrays (Agilent 44K and 244k), mRNA expression microarray (Affymetrix U133 plus 2.0), verified by quantitative real-time PCR (RT-qPCR), next-generation sequencing (NGS), miRNA microarray expression (Agilent 60K), Western immunoblotting, and the comet assay DNA damage test.

The *FANCA, FANCB, BRCA1* and *BRCA2* showed significantly changed ($p<0.05$) DNA methylation levels in LSCC cell lines. Although, only *FANCA* was showed with significant difference (hypomethylated) in LSCC primary tumors ($p<0.0001$), what was also shown in tumor margin samples ($p<0.0001$) and *LINE-1* and suggests that DNA methylation is globally lowered in LSCCs. The *FANCA*-hypomethylated LSCC patients had better (2-fold) overall 5-year survival by comparing methylation quartiles ($p=0.004$) and by arbitrary cut off at 60% of methylation ($p<0.001$). The mRNA microarray expression data showed significant ($p<0.05$) overexpression in 6 FA-related genes (*FANCA, FANCB, BRCA1, FANCI, FANCL* and *BRIP1*). The qRT-PCR displayed significant overexpression of *FANCA* in both, LSCC cell lines and primary tumors ($p<0.005$) but no linear correlation with methylation ($p=0.85$) was found. The array-CGH data (Agilent 244K and 44K) showed minor chromosomal

imbalances of FA loci in LSCC cell lines. The *miR-940, -374b, -1246* and *-1290* were the only differentially expressed ($p<0.05$) and unreported in HNSCCs. The *miR-940* targets the *FANCA* and upregulation of *miR-940* could affect *FANCA* function. Further, the NGS delineated no exonic mutations except SNPs (one possibly damaging, rs17233497). The Western blot resulted in diversified FANCA protein signal. Regarding FANCD2 protein only two cell lines were showed (UT-SCC-34 and UT-SCC-50) with both FANCD2 forms, L and S. Further, according to the FANCA protein level four cell lines (with no FANCA protein, high FANCA protein level and two with moderate level) were subjected to comet assay. All cell these lines demonstrated different levels of nuclei DNA expansion which directly corresponded to DNA damage level.

The low *FANCA* methylation of tumor cells was associated with better overall survival among LSCC patients. This result is in opposition to the theory, where low methylation is translated into higher expression of *FANCA*, causing better response to DNA damage (chemotherapy) and finally worse prognosis due to favored tumor growth. However, the distortion presented here is possible by specimen impurity or by other not analyzed FA-related factors. Alternatively, *FANCA* upregulation can be induced by DNA damage through *ATR* and *CHEK1*, also overexpressed in LSCC cell lines.

The FANCA protein levels suggest its expression in a portion of LSCC cell lines. Nonetheless, the lack of FANCD2 monoubiquitination indicates probable inactivity of the FA/BRCA pathway in most of analyzed LSCC cell lines. Undescribed in HNSCCs *miR-940* may have influence in *FANCA* regulation. They could affect translation, explaining the discrepancies between mRNA and protein levels of the *FANCA* gene.

Notwithstanding, the FANCA protein level was involved in DNA repair potential in the few cell lines treated with an alkylating agent (cisplatin). Considering FA/BRCA pathway as inactive, an alternative and independent role of FANCA protein can probably function apart from the rest of the FA-related proteins.