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Department of Oncology-Pathology

Molecular characterization of phenothiazines in experimental cancer therapy – New tricks of an old drug revealed

AKADEMISK AVHANDLING
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ABSTRACT

Cancer is characterized by uncontrolled malignant proliferation of cells that eventually interfere with tissue/organ functions. Traditionally, cancer is treated with chemo- and/or radiotherapy when surgery is not an option. Unfortunately, the efficacy of conventional anti-cancer chemotherapy is severely limited by therapy resistance. A conceptually appealing strategy to combat tumor resistance is to use chemosensitizers, compounds that selectively sensitize tumor cells to chemotherapy without affecting normal tissue. Phenothiazines belong to a class of "old" drugs that are used clinically to treat psychiatric disorders. In this thesis, we characterized the chemosensitizing potential of phenothiazines in combination with DNA damaging chemotherapeutic drugs. Our primary aims are to elucidate the molecular mechanisms by which phenothiazines impart sensitization and to delineate molecular determinants that predict responsiveness of tumors to phenothiazine-based intervention. In **Paper I**, we confirmed that the phenothiazine compound trifluoperazine (TFP) was a potent sensitizer of bleomycin in human non-small cell lung carcinoma (NSCLC) cells; the likely mechanism being inhibition of repair of DNA single strand breaks (SSB) as well as DNA double strand breaks (DSB). In **Paper II**, we found that TFP delayed the resolution of bleomycin- or cisplatin-induced γH2AX, a marker of unrepaired DNA DSB, prolonged the cell cycle arrest and increased oxidative stress in NSCLC cells. TFP co-treated cells eventually resumed cycling without fully repairing the DNA damage, which led to mitotic defects, secondary checkpoint arrest, exacerbated oxidative stress, organelle dysfunction, caspase activation and ultimately apoptosis. In **Paper III**, we uncovered a possible link between phenothiazines and chromatin remodeling by *in silico* gene expression analysis. We found that TFP and structurally related phenothiazines significantly enhanced the activity DNA-PK/ATM in tumor but not normal fibroblasts in response to DNA DSB-inducing agents, resulting in increased selective phosphorylation of a subset of ATM substrates with chromatin regulatory functions. Notably, this represents an adaptive response which could be targeted by DNA-PK/ATM inhibitors to further enhance TFP-mediated chemosensitization in NSCLC cells. Moreover, we found that wild-type p53 is a potential predictor of unresponsiveness to phenothiazine-based chemosensitization. We further demonstrated that TFP preferentially increased the cytotoxicity of direct-acing DNA damaging agents, but not indirect-acting DNA damaging or non-DNA damaging agents, in p53-deficient tumor cells (NSCLC, breast cancer). In **Paper IV**, we compared the gene expression profile of NSCLC residual clones that survived cisplatin treatment with counterparts that survived cisplatin/TFP co-treatment. We found that survival after cisplatin was associated with enrichment of pathways involved in DNA metabolism/repair, cell cycle and RNA post-translational modification. Pathway analysis showed that several DNA repair genes were concurrently up-regulated in residual clones that survived cisplatin treatment, but not in residual clones that survived cisplatin/TFP co-treatment did not shown such enrichment of DNA repair genes. In summary, our data showed for the first time that inhibition of DNA DSB repair by TFP is related to alterations in DNA-PK/ATM signaling, which led to increased apoptosis in the short term and gene expression changes as well as loss of clonogenicity in the long term. Further, our identification of molecular contexts that predict responsiveness to phenothiazines will aid in the design of future clinical trials.

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