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Measurement of sensitivity to DNA damaging agents

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i salen Kammaren på Sahlgrenska Universitetssjukhuset, fredagen den 8 September 2017, klockan 09:00

av Sherin T Mathew

Fakultetsopponent:
Elias Arner, Professor, PhD
Karolinska Institutet, Stockholm, Sweden

Avhandlingen baseras på följande delarbeten

I. **Sherin T. Mathew**, Pegah Johansson, Yue Gao, Anders Fasth, Torben Ek, Ola Hammarsten. A flow cytometry assay that measures cellular sensitivity to DNA-damaging agents, customized for clincal routine laboratories, Clinical Biochemistry, 2016, 49:566–572.

II. Pegah Johansson, **Sherin T Mathew**, Michaela Johansson, Ola Hammarsten. Validation of Cell division (CD) assay in measuring sensitivity of Fanconi Anemia cells. Manuscript, 2017

III. **Sherin T. Mathew**, Petra Bergstrom, Ola Hammarsten. Repeated Nrf2 stimulation using sulforaphane protects fibroblasts from ionizing radiation, Toxicology and applied pharmacology, 2014, 276: 188-194.

IV. **Sherin T. Mathew,** Ola Hammarsten. Preconditioning cells with sulforaphane or bardoxolone methyl induces adaptation and cross-adaptation in human skin fibroblasts**.** Manuscript, 2017

SAHLGRENSKA AKADEMIN
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Measurement of sensitivity to DNA damaging agents

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Abstract

There is a large inter-individual variation in intrinsic sensitivity in patients receiving treatment with DNA damaging agents. Cancer therapy exemplifies this problem where patients experience varying degree of normal tissue side effects in response to radiation or chemotherapy. For this reason, it is necessary to develop an assay to predict sensitivity of a patient prior to treatment with DNA damaging agents. This may allow more individualized treatment and improve the therapeutic index. In paper I and II we focused on developing and validating a flow cytometry - based cell division assay (CD) that uses the thymidine analogue EdU (5-ethynyl-2'-deoxyuridine) to measure the proliferative ability after DNA damaging treatment. In paper I, the CD assay measured sensitivity to radiation of human skin fibroblasts with a correlation similar to the standard clonogenic survival assay in a relatively short time frame. Using the easily sampled peripheral blood lymphocytes, the CD assay found variation in intrinsic sensitivity to radiation and detected increased sensitivity in patients with DNA repair defects. In paper II, the CD assay was further validated for measurement of cell sensitivity to DNA damaging drugs. The results indicated that the assay can be used to identify sensitive patients.

Exposure to ionizing radiation generates free radicals that carry out most part of the toxic effects. The cellular antioxidant system regulated by the Nrf2 transcription factor plays a key role in protecting cells against radical induced damage; hence in paper III we have investigated if pretreating cells with Nrf2 activators influence the sensitivity to radiation. Results from paper III demonstrated that repeated treatment using the isothiocyanate sulforaphane protected human skin fibroblasts from toxic effects of ionizing radiation in an Nrf2-dependent manner. In paper IV we found that repeated pretreatment of cells with Nrf2 activators, sulforaphane or synthetic triterpenoid bardoxolone methyl trained the cells to acquire resistance against higher toxic concentrations of both drugs. Together these results indicate that repeated stimulation of Nrf2 system can enhance cytoprotection and that adaptation to stress may be a general feature of the Nrf2 response mechanism.

Keywords: [Intrinsic sensitivity, DNA damage, ionizing radiation, cell division, Nrf2, sulforaphane, bardoxolone methyl, cytoprotection]

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