358 Poster

Gonadotropin signalling in ovarian cancer cells - roles of calcium, PKC and Pyk2 in ERK1/2 activation

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The majority of ovarian tumours occur in postmenopausal women when levels of the serum gonadotropins follicle-stimulating hormone (FSH) and luteinising hormone (LH) are high. Activation of mitogen-activated protein kinases (MAPKs) plays a key role in mediating cellular responses such as proliferation in response to various extracellular stimuli including hormones and has been described in ovarian cancer. Therefore, the aim of this study was to identify which cell signals lead to initiation of the MAPK cascade by gonadotropins in order to understand the molecular mechanisms of gonadotropin action in ovarian tumours. Ovarian epithelial cancer cell lines (OV167, OV207, PE01, OVCAR-3) were treated with 10 nM FSH or LH in a time-dependent study and subjected to immunoblotting. Results showed an increase of phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) at 5-15 min. Treatment with EGTA and dantrolene, an inhibitor of Ca^{2+} release from intracellular stores, abrogated phosphorylation of ERK1/2 as did treatment with the L-type voltage-gated calcium channel blocker verapamil, indicating dependence on both intra-and extracellular Ca^{2+}. Furthermore, gonadotropin induced ERK1/2 phosphorylation was dependent on protein kinase C (PKC) as demonstrated by inhibition of ERK1/2 phosphorylation with the PKC inhibitor GF109230X. The proline-rich tyrosine kinase 2 (Pyk2), a cytoplasmic nonreceptor tyrosine kinase related to focal adhesion kinase, responds to stimuli which elevate intracellular Ca^{2+} and is known to activate the MAP kinase signalling pathway via PKC. In response to treatment with LH and FSH, the Pyk2 autophosphorylation / Src kinase binding site at Tyr-402 was phosphorylated, suggesting that Pyk2 might be involved in FSH and LH induced MAPK signalling. Taken together, these results show that gonadotropins trigger MAPK signalling in a calcium-dependent manner involving PKC and possibly Pyk2. This data provides new insight into complex gonadotropin signalling events in ovarian cancer cells.

359 Poster

Control of the G2/M checkpoint after exposure to low dose of ionising radiation - implications for hyper-radiosensitivity

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Following DNA damage, cells undergo G2/M cell cycle arrest, allowing time to repair prior to mitosis. Two molecularly distinct G2/M checkpoints are induced by ionizing radiation: the early checkpoint that represents the failure of cells which had been in G2 at the time of irradiation to progress into mitosis and G2/M accumulation that occurs later and represents the accumulation of cells that had been in earlier phases of the cell cycle at the time of irradiation. While molecular mechanisms of G2 accumulation are now almost fully elucidated, the pathways involved in the control of the transition between G2 and mitosis remain to be identified. Evading this early checkpoint was suspected to lead to hyper-radiosensitivity (HRS). G2/M checkpoints are essential for cell survival and maintenance of genomic stability after irradiation, so an essential question is if there is a threshold dose for the activation of these checkpoints.

In order to answer this question and determine molecular mechanisms underlying G2 phase arrest, we investigated the response to low doses of ionizing radiation of two glioblastoma cell lines exhibiting or not HRS, including consequences of ATM and PARP proteins activity modulation.

Our results show that G2 accumulation is effective for doses as low as 0.2 Gy and is dose-dependent between 0.2 and 2 Gy. ATM inhibition does not interfere with radiation-induced G2/M block but PARP inhibition does. Prevention of G2 to mitosis transition after irradiation occurs only above a treshold dose varying with the cell line and requires ATM activity. Moreover, our data lend support to a direct correlation between the early G2/M checkpoint and the ability to exhibit HRS.

Our studies provide evidence that exposure to low doses of ionizing radiation lead to the activation of the two G2/M checkpoints described for higher doses, both in a dose-dependent manner but with distinct threshold and involving distinct damage signalling pathways.

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360 Poster

ATF3 and p15PAF are novel gatekeepers of genomic integrity upon UV stress

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Environmental genotoxic stress is one of the major causes of genomic instability. During such a stress, the maintenance of genomic integrity is of crucial importance to allow proper cellular functioning. Importance of this process is underscored by the numerous human syndromes, such as Xeroderma pigmentosum (XP), linked to mutation of genes involved in DNA repair. XP patients are highly photosensitive and prone to develop skin tumors from keratinocyte origin such as basal and squamous cell carcinoma. Proper DNA damage response is therefore of great importance for UV inducible anti-cancer barrier that elicits growth arrest, repair or cell death. Following UV stress, normal cells trigger induction of numerous transcription factors such as activating transcription factor 3 (ATF3). Induction of ATF3 often correlates with cellular damages suggesting an important role during the cellular stress response. In this study, we show that repression of the transcription factor ATF3 following UV mediated genotoxic stress impairs the DNA repair process. We provide evidences that ATF3 directly regulates the PCNA-associated factor KIAA0101/ p15PAF. We further demonstrate that ATF3 and P15PAF gene expression are sufficient to trigger DNA repair machinery and lack of their expression alters the nucleotide excision repair pathway. We show that overexpression of p15PAF compensates lack of ATF3 expression thereby constituting a major effector of ATF3 in the DNA repair process. Finally, impairment of DNA repair, due to the lack of p15PAF expression, rendered the cells more sensitive to UV induced cell death. Our results suggest ATF3 and p15PAF as novel gatekeepers of genomic integrity following UV exposure.

361 Poster

Acquired cisplatin-resistance with increased caspase-3 activity but suppressed caspase-8 and -9 activity

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Resistance mechanisms, including changes in apoptotic signalling pathways, are important limiting factors in the treatment of solid malignancies with cis-diamminedichloroplatinum(II) (cisplatin). To study the effects of acquired cisplatin-resistance on apoptotic caspase activity, a human malignant pleural mesothelioma cell line (P31) was compared to a sub-line (P31res1.2) with acquired cisplatin-resistance. Materials and methods: Caspase-3, -8, and -9 activities were determined with fluorescent activity assays after 0.5, 2, 6 and 24 h of exposure to cisplatin-free or cisplatin-containing medium. Equitoxic cisplatin concentrations were used, 10 mg/L for P31 and 40 mg/L for P31res1.2 cells. Caspase cleavage and expression of caspase substrates and proteins of the intrinsic apoptosis signalling pathway were determined with Western blotting, and DNA fragmentation was determined with FACS analysis of TUNEL-stained cells. Results and discussion: The P31res1.2 cells, that are four-fold more resistant to cisplatin than the P31 cells, had a higher basal activity of caspase-3 and increased expression and cleavage of caspase-8 and -9, although the basal caspase-8 and -9 activities were similar to those in P31 cells. In addition, the P31res1.2 cells had increased expression of the pro-apoptotic proteins Apaf-1, cytochrome C and HtrA2/Omi. However, cisplatin exposure increased the cytochrome C expression and decreased the XIAP expression in the P31 cells but not in the P31res1.2 cells. Despite this, the maximal activity of caspase-3, -8 and -9, and the extent of DNA fragmentation, was similar in both cell lines, indicating that caspase-8 and -9 activity was suppressed in the P31res1.2 cells, which delayed the initiation of apoptosis despite the increased basal caspase-3 activity. There was very little caspase-8 activity in both cell lines; instead, the main cisplatin target was the mitochondrial apoptotic signalling pathway. Conclusions: This study shows that acquisition of cisplatin-resistance can result in higher basal caspase-3 activity, earlier cisplatin-induced caspase-3 activation, increased expression and cleavage of caspase-8 and -9, and that XIAP can have a role in cisplatin-resistance without being over expressed. Caspase expression, caspase cleavage and XIAP expression are used as markers of apoptosis and chemotherapy resistance in patient material, and these results emphasise the importance of careful interpretation of such data to avoid false conclusions.