MARIE CURIE ACTIONS

**Incoming International Fellowships (IIF)**

**Call: FP7-PEOPLE-IIF-2008**

**Final report**

**Project Acronym:** IGF1RHC

**Project Code**: **237785**

**Project title**: Targeting IGF-1 receptor in liver cancer with focus on its mechanistic role in transcription and its interaction with the cell cycle machinery

**Overview of the results**

The insulin-like growth factor-1 receptor (IGF-IR) is currently being a promising candidate for cancer treatment and several clinical trials with anti-IGF-IR compounds are in progress. Compounds targeting IGF-IR include anti-receptor antibodies, anti-ligand antibodies, receptor-specific tyrosine kinase inhibitors, and agents such as picropodophyllin (PPP) that have novel mechanisms of action not yet fully elucidated. Surprisingly, specific anti-IGF-1R antibodies have not shown any clinical benefits, neither as mono-therapeutic option nor in combination with conventional chemotherapies. In contrast, a phase I/II clinical trial has shown that PPP, as a mono-therapeutic option, is well tolerated and tends to increase survival in patients with non-small cell lung cancer (NSCLC) ([Ekman](#_ENREF_2) et al., 2010). This raises the question of whether PPP mediates off-target effects that may contribute to its anti-tumour efficacy but at the same time do not generate any serious adverse effects. It is, therefore, important to study the mechanism of action of such a promising anti-cancer drug currently in clinical trials. Among the anti-cancer effects of PPP cell cycle phase arrest and apoptosis have been previously reported. In the present study we focused on the detailed characterization of how PPP affects the cell cycle and kills cancer cells? We used a panel of different cancer cell lines and in vivo xenograft cancer model in comparison to normal cells.

**Objectives**

The project had two objectives:

1. Study the role of IGF-1R in transcription in malignant cells.
2. Study how IGF-1R modulates the cell cycle machinery?

**Results**

**For objective two**

1. **To assess the role of IGF-1R in cell cycle regulation and in apoptosis**, we treated HepG2, Hep3B and Huh7 liver cancer cell lines with siRNA targeting the IGF-1R or with IGF-1R inhibitors such as NVP-AEW541 or PPP and measured cell proliferation, cell cycle and apoptosis using Annexin V/Propidium iodide before and after the addition of IGF-1. We also studied the expression of the following proteins before and after siRNA of IGF-1R: pIGF-1R, IGF-1R, pAKT (T309), pAKT (S473), pERK, ERK, pCDK1Y15, pCDK1T161, CDK1, pCDC25C, CDC25C, pChk1, Chk1, pChk2, Chk2, p53, p21 and p16. We found slight reduction in apoptosis after the addition of IGF-1, however, no alterations in the expression of the above mentioned protein was observed after the knockdown of IGF-1R.
2. **How PPP induces G2/M arrest and apoptosis in cancer cells**

* In the present project we studied in detail the effect of PPP on (**A**) a number of human cancer cell lines (HepG2, Hep3B, Huh7, MCF-7, A549 and U2OS cells) in comparison to normal hepatocytes (nHeps), (**B*)*** *Cdk2-/-* mouse embryo fibroblasts, **(C)** Mouse xenograft model of lung cancer. We studied the following parameters: (1) cell proliferation, (2) cell cycle distribution, (3) apoptosis, (4) CDK1 activity, (5) protein expression and phosphorylation of cell cycle and apoptosis regulators, (6) mRNA expression of cyclin B, cyclin E and p53, (7) microtubules. We confirmed previous studies that PPP inhibits proliferation and induces apoptosis in cancer cell lines. We uncovered that the previously described G2/M arrest induced by PPP is in fact a mitotic arrest in prometaphase. We confirmed this using time-lapse video microscopy and fluorescence microscopy. Mitotic arrest occurred as early as 4h after treatment in some cell lines. It was associated with a pronounced increase in CDK1 kinase activity. Further analysis showed that cyclin B protein levels and the CDK1pT161 were also increased. Similar results were obtained in xenograft lung cancer model. Cyclin B mRNA was also upregulated with similar kinetics. At late time points p53 and p21 protein levels were increased in cell lines. Regarding the apoptosis-regulating proteins PPP treatment resulted in a decrease in Mcl-1, Bcl2, Bax and cleavage of PARP. In order to confirm our data we knocked down CDK1 with specific siRNA in MCF-7 cells and treated the cells with PPP. CDK1 knockdown could rescue the mitotic arrest, apoptosis and the associated alterations in protein levels. It is noteworthy to mention that PPP induced the above mentioned alterations only in cancer cell lines and did not affect normal hepatocytes or normal lung tissue from mice with lung cancer xenograft. PPP did not have an effect on MEFs deficient for Cdk2 and did not have a direct effect on CDK1 in a cell-free system. PPP modulated microtubule dynamics in liver cancer cell lines only after 24 h of treatment in comparison to colchicine. Taken together, the effect of PPP on cell cycle and apoptosis, restricted only to cancer cells and not normal cells, involves CDK1 activation.

For objective one: Role of IGF-1R as a transcription factor in malignant cells

We have transfected Huh7 cell lines with a construct containing full length wild type IGF-1R (WT-IGF-1R) and another construct containing IGF-1R with the triple SUMO- mutations (TSM-IGF-1R). We performed cellular fractionation and could detect the IGF-1R-TSM in the nuclear portion, which made us switch to a new construct with a FLAG tag to be able to differentiate the exogenous nuclear IGF-IR from the endogenous. In parallel, in collaboration with another group we tried to stably express the WT-IGF-1R and TSM-IGF-IR in R- cells (cells deficient for IGF-1R) using viral vectors. We experienced technical difficulties because the efficiency of transduction was much higher with the WT-IGF-IR and no transduction with the TSM-IGF-1R took place. We are currently trying to modify the experimental system to overcome the technical difficulties. We have not further worked on this objective due to the reasons mentioned above.

**Conclusion**

The results from the present project provided more insight into the mechanisms of action of PPP, a promising anticancer drug currently in clinical trials, and into its efficacy in treating experimental liver and lung cancer. The antitumor effects of PPP were selective for cancer cells and did not affect normal cells.

**Socio-economic impact of the project**

This project had a significant socioeconomic impact on liver cancer research especially in Egypt. Through this project we started new collaborations with groups working on liver cancer in Egypt and in Heidelberg Germany. As a result of this project three young Egyptian researchers received training at KI under supervision of Dr Aleem. Since liver cancer is not very common in Sweden we could also confirm our findings in lung cancer (the current Phase I/II clinical trials are on lung cancer) and created a network of collaboration with other investigators at KI.