ERASMUS STUDENT MOBILITY PLACEMENT FINAL REPORT

Motivation to participate in Erasmus programme

My interest in Biology and science is extremely strong and a great part of my life. I have been studying Biology since Primary School but I became aware of my interest during Secondary education. From that moment on I have been focusing on improving my knowledge, not only in class but in my free time as well. Even though there are numerous obligations and classes in my University course, there is still enough time for me to be a part and even the initiator of scientific events promoting Science. When biology is in question, I am mostly interested in Cancer biology and Epigenetics. Since the programme of my studies has been really wide, the graduate programme at the Faculty of Biology LMU was the time to choose the field of studies and use the acquired knowledge in the best possible way. Always being motivated, I have decided to continue my studies towards the PhD programmes. This required an appropriate search for a suitable Institution where I can practice research work before applying to the programme itself. Institute Curie in Paris, France has intrigued me as a place where I can gain the skills and experience necessary for my future research work. High quality of internships and research at the Curie institute made my decision an easy choice and I have decided to apply there and as part of internship to do also my Master thesis. Especially I like international environment of the Institute and the fact that I'll be spending quite some time abroad learning new cultures and meeting different people. This would give me an opportunity to improve my English skills and also to learn complete new language as well. I am always ready to learn more and broaden my perspective. Through my Master Progamme of Biology at LMU I've spent most of the time doing research work together with the Lectures and Seminars offered within the programme. Attending those classes I was able to see that working in international labs was the best and most efficient way of learning not just science but also from another prospective.

This internship will enable the Masters degree which is very important or even essential for my future studies. Through these programmes I would get a great opportunity to improve my knowledge and acquire practical skills which would give me wide options in my future scientific career. The experience I would be able to acquire from the courses during the time spent in Erasmus+ internship programme would create a perfect media for the international scientific carrier I am hoping to achieve.
THE HOST ORGANISATION

Host organization: Institut Curie

Country: France

Field of the host organization: Cancer research

Application process

At the time of application for the Erasmus+ grant I was in my last semester of 2 years Master programme in Biology at the LMU. Most of students in my programme are working on their Master's thesis as final step necessary to obtain the Diploma. As we were allowed to do the Master Thesis work outside the LMU, in my search for suitable Institutions where I could carry out the Thesis, I've came across the Institut Curie. Institut Curie has long and good tradition of teaching the Master students as part of either Master thesis or shorter internships. I've applied directly to the lab where I was interested in the research they were doing. Laster it was more the easy through consultation with both my student coordinator Dr. Boegle and erasmus coordinaton Mr. Johannes Hoch to participate in the Erasmus+ programme while working on my Thesis abroad.

Integration of new people and life at the campus

When I arrived at the Institut Curie I was welcomed nicely and official integration went without any problem. Institut Curie is located at many sites in Paris and outside Paris. I've spent my time at Institut Curie Orsay site which is big campus of different science faculties under the Paris Sud University. Campus itself has everything what students can expects. I was given student mensa card that enabled me to eat at discounted prices and the traffic connections were good from home to the Campus. Institut itself is highly international one and I was able to navigate everything with just speaking in English language. I've met many people from different countries that were doing the internship at the same time as I was doing. I've participated in the regular lab meeting, unit meeting and journal clubs. We were give the possibility to attend all the scientific talks and seminars that took the place at the site. I was offered to take part in preparative French course and was really satisfied with the basic skills I've learnt in just few months.
Housing

Finding a place to stay during the internship time can be stressful, especially if you are coming from abroad and you are finding a place to stay in cities with competitive market such as Paris. Before my internship started I was transferred emails from the other Institut members containing information how to find and also about available apartment close to the Institute. Institute also advised to use some of the agencies that operate in Paris. I've decided to take in charge Lodgis agency. Without any problem I was able to find and english speaking real estate agent that found me an apartment and led me through all the process of signing a contract, finding appropriate insurance policy etc.

Tasks performed during the work placement

Preliminary data from the host laboratory has identified two residues, C315 and S273 that when mutated, affect DNA binding of the N-terminus of BRCA2 whereas G267E does not affect DNA binding but confers a cytokinetic defect to the cells. Thus, this internship will be focused on characterizing the phenotype of cells carrying these variants identified in breast cancer patients and located in the newly discovered DNA binding domain of the N-terminal region of BRCA2.

1. Generation of stable cell lines carrying C315S, S273L and G267E variants in the BRCA2 deficient human cell line DLD1. (1-2 months)

2. Characterization of DNA repair proficiency of these cell lines using in vivo homologous recombination assay. (1 month)

3. Clonogenic survival of BRCA2 mutated cell lines upon different DNA damaging agents: MMC, IR. (2 months)

4. Based on the results from 2 and 3 and the time left at this point of the internship, we will conclude the characterization of these variants using a: videomicroscopy to analyse a possible cytokinetic defect as observed in G267E or b: Immunofluorescence microscopy to analyse the effect of the variants on DNA repair foci formation. (1 month)
Obtained results during the placement period

Deficiency in BRCA2 function is associated with defective HR. My results show that cells carrying mutations C315S and S273L, located within the NTD of BRCA2, show a reduction in homologous recombination in a HDR in vivo assay. These results are consistent with previous experiments in which we showed that the C315S mutated NTD reduces the promotion of RAD51 DNA strand exchange activity in vitro. Importantly, my results of BRCA2 C315S and S273L stable cell lines indicates that HR is severely reduced in the context of the full length BRCA2 in cells in which the CTD is intact and functional. Although very encouraging, these results need to be further confirmed with at least one more stable clone of each mutation. A second round of the stable clones generation is ongoing and after expression levels are confirmed it will be used as confirmation of the results shown here. If possible, it would be interesting to attempt introducing those mutations BRCA2 using the CRISPR-Cas9 system. However, since BRCA2 is an essential gene, its disruption may lead to cell lethality. BRCA2 deficient cells are defective in Homologous recombination of the double strand breaks. Deficient BRCA2 cells are hypersensitive to DNA interstrand crosslinks caused by mitomycin C. Both C315S and S273L mutant showed sensitivity to the MMC treatment. In particular S273L mutant was sensitive almost to the level of BRCA2 deficient cells (DLD1/-/-). It is possible that the CTD and the NTD have evolved to repair specific types of DNA damage. To test this hypothesis, in future work we will analyse the cell survival upon various DNA damaging agents such as Poly (ADP-ribose) polymerase (PARP) inhibitors. Although PARP1 is an enzyme mainly involved in the repair of single strand breaks (SSBs), these lesions may be converted on DSB during replication, thus PARP inhibition results in the cell death in BRCA2 deficient cells. Regarding my second objective on BRCA2 dimerization, I was able to generate red-tagged BRCA2 at the C-terminus, a tool that will be important for our future experiments to investigate possible BRCA2 dimerization. At first, we will do co-immunoprecipitation of two differently tagged BRCA2. I have already transfected the red-tagged construct in the stable DLD1 -/- cells expressing wild type GFP BRCA2 and everything is set up to perform co-Immunoprecipitation using GFP-trap. If BRCA2 forms a dimer, we should be able to pull down the BRCA2-RFP bound to GFP-BRCA2. In a next step, and if the complex is confirmed, we will test which is the active oligomeric state of the BRCA2 by monitoring the possible dimerization of BRCA2 at induced the DSBs. For this, I have optimized the visualization of gH2AX foci at induced DSB using the AsiSI tamoxifen inducible system31 in U2OS cells. This system generates around 100 breaks at specific sites. We will visualize BRCA2 dimers by co-localization of Proximity ligation assay (PLA) spots using antibodies against GFP and RFP or FRET, for which GFP and RFP are a very effective pair. This system should allow us to monitor the possible enrichment of dimers of BRCA2 at the p-gH2AX positive sites.
My final conclusions

The time I've spent in the Erasmus+ internship enabled me to finish my Master studies at the LMU. In the time of this internship I've also got my PhD position at the Institut Curie. I fulfilled all of my expectations. I'm particularly happy and thankful that I was able to spend one semester abroad, meeting new people and cultures, learning totally new language for me and practising even better my English skills. Spending six months period in Paris was indeed amazing. I had many benefits as a students and was able to enjoy all the sightseeing and visiting different museums and galeries for free as a young (under 26) EU citizen. I would like to thank to all the people from LMU and my host Institution that made this as a Semester to remember.