COST STSM final report

Details of the STSM

Title: Exploring the noncoding RNA function in stemness features of HPV positive cancers. **CA18117**

Start and end date: 17/04/2023 to 17/07/2023

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section. 500 word

Human papillomavirus (HPV) is a sexually transmitted high-risk virus for its oncogenic potential in inducing cervical cancer. Nevertheless, its tumorigenic ability is not limited to this site. Laryngeal cancer (LCa), considered quite rare in women, is for example strongly related to this infection. In recent years, there is an increasing interest in the role of the non-coding RNA for their diagnostic, prognostic potential, and their biological effect as tumorigenic or tumour suppressor agent. Recent studies have underlined the overexpression of miR-223-3p in serum and tissue of LCa patients as well as an involvement of miR-223 in the carcinogenesis of cervical tumour by the axis STAT3-miR-223-TFBR3/HMGCS1. Further studies on miR-223 suggest that it triggers both Epithelial to Mesenchymal Transition (EMT) and angiogenesis. This STSM, which was carried out in as part of GYNOCARE COST Action , was focused on the study of a possible connection between miR-223-3p overexpression and HPV positivity in activating stemness associated genes. This was tested by using a specific laryngeal carcinoma (LCa) in vitro model. Specifically, the interaction between oncomiR miR-223 and HPV in LCa was studied focusing on the modulation of specific genes related to the stemness signalling pathways. HEp-2 and HNO-210 cell lines represented the LCa in vitro model that we selected for this study. The two cell lines were respectively positive and negative for HPV. These cell lines have been previously transduced to overexpress or inhibit miR-223 expression through stable lentiviral infection. To this aim, an extensive bioinformatic analysis on the predicted miR-223-3p targets was performed on different online tools, such as mirtargetlink 2.0. This analysis highlighted a large number of genes that can be potentially deregulated by the action of the miR-223 and that can act as a point of connection with the signaling pathways HPV-related. Amongst a copious list of them, the gene F-box/WD (FBXW7), famous for its antiproliferative and tumor suppressor action, presented a high prediction score. Moreover, FBXW7 is known to inhibit the epithelial-to-mesenchymal transition. For this reason, a specific Epithelial to Mesenchymal Transition array profiling for simultaneous detection of 48 genes was performed on HEp-2 (HPV positive) cells transduced to overexpress or inhibit the miR-223. The Hep-2 miR223 mimic cells showed a down-modulation of the gene (CAMK2N1) and up-regulation of 3 (COL3A1, DSC2 and TCF4) genes when compared with their corresponding Negative Control, while the HEp-2 cells, transduced with miR-223 inhibitor showed up-regulation of 3 genes (CDH1, GNG11 and IGFBP4) when compared with their corresponding Negative Control. Then, functional in vitro assays such as proliferation, migration were performed to assess the effect of miR-223 in the modulation of EMT.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section. (max. 500 words)

This STSM aimed at displaying the involvement of miR-223 in stemness of HPV-related cancers comparing to what is known to cervical cancer stem cell establishment and development. This is in line with the objectives proposed by the GYNOCARE COST Action, specifically WG1 (Basic research). LCa is, in fact, a rare tumour in women and the correlation between HPV and the LCa has not yet fully understood above all regarding the stemness. The latter is a very important factor for chemo-resistance and immunological escape of squamous cancers. This is the most frequent histotype found in both cervical and laryngeal cancers and share common pathological and molecular features. In this way, the correlation between molecular alterations found in HPV-related LCa was associated with those in cervical cancer. The investigation on the biological processes and signalling pathways active in cancer stem cells in HPV-related cancers is useful in order to target the stem cell population in a cancer and to strengthen the therapeutic strategies in these tumours. During this STSM, I have demonstrate how an involment of the miR-223 in the HPV positive cells is related to the activation of EMT, one of the mechanisms displayed by stem cell population in different cancers. This molecular evidence was also confirmed by *in vitro* migration assay, where the Hep-2 miR-223 mimic cells showed an increasing migratory capacity at different time points in comparison to the negative control. These preliminary findings should be further investigated to assess the impact of the miR-223 in cancer stemness status of HPV correlated cancers. The STSM was a strong personal and scientific growth for me, and, at the same time, it was a wonderful opportunity to understand more deeply a molecular pathway activated in HPV-positive cells. The study we conducted aimed to provide the basis for prospective studies of targeted therapy against potentially elucidated targets. Thus, during the three month period I was not only working in the laboratory environment but also meeting new people and making new connections from all over the world with other researchers who were interested in the various scientific topics. Moreover, as a woman and young researcher I totally appreciate the GYNOCARE objectives, and I feel honoured to be part of this worthy Action. This international collaboration has reflected one of the Working Group One objectives, which is to build a network of researchers involved focusing on rare diseases. Thanks to GYNOCARE it has been possible to investigate the HPV role in stemness of rare tumours for women, such as LCa making a comparison with cervical cancer.