Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA18117

Grantee name: Chiara Tammaro

Details of the STSM

Title: Displaying the Link between HPV Cervical Cancer and Laryngeal Cancer throughout the involvement of miR-223: a Potential Biomarker

Start and end date: 30/04/2022 to 19/07/2022

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.

(max. 500 words)

Grantee enters max 500 word summary here.

The STSM, which was carried out in the largest gynocare mission, had as a goal the identification of the possibly existing correlation between the miR-223 and HPV in laryngeal carcinoma (LCa). This tumour is quite rare in women, and it has been seen a correlation with the presence of the HPV to be further investigated. Moreover, from our preliminary data, we found that the expression level of circulating miR-223 was significantly upregulated in serum of LCa patients. In addition, miR-223 has been reported being involved in the carcinogenesis of cervical tumour.
For this reason, during the STSM the interaction between the oncomiR miR-223 and HPV in LCa was studied focusing on the activation or the inhibition of specific target proteins.
The LCa *in vitro* model selected for this study is represented by HEp-2 and HNO-210 cell lines, which are respectively positive and negative for HPV. These cell lines were previously transduced to overexpress or inhibit miR-223 expression through lentiviral infection.
The activities of the STSM started with the search for the similarities shared by the cervical carcinoma and LCa. One of the most evident points of connection between those two cancers is the squamous nature of the tumour cells. In addition, from research carried out on "The Human Protein Atlas" and other similar databases, both tissues of cervical carcinoma and LCa present a highly level of expression of the Transglutaminase 2 (TG2), a trans-peptidase, mainly modulated by Ca^{2+}, with a wide localisation, which plays a multifunctional role in the biochemical functions and in many diseases. In details, in the cervical carcinoma, TG2 is highly expressed in HPV positive low grade premalignant lesions of the cervix, suggesting its role in the transformation of the malignancy. TG2 forms a stable ternary complex along with fibronectin and collagen, which is very important for maintaining the cell position and regulating cell migration. In the specific cancer environment, TG2 enables tumour cells to escape the apoptosis by promoting the activation of nuclear factor kB (NF-Kb) pathway, which is notoriously involved in inflammation and in oncogenesis. As a result, this activates the metastasis progression and an increasing

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

cell proliferation. Moreover, it was reported also a positively correlation amongst miR-223 and NF-Kb. On this basis, this scientific evidence was used to remodulate the experimental design of the STSM. The hypothesis was to underline a cooperation between TG2-NFKB activation and miR-223 in the previously described *in vitro* model. To this aim preliminary western blots were performed in order to study the level of expression of TG2 in the different cell lines and the activation of NF-kB pathway. Then, functional *in vitro* assays such as proliferation, migration and invasion assays were performed to assess the effect of miR-223 in relation to the relative expression level of the studied proteins in HPV positive or negative cells. In addition, a proteomic analysis of the *in vitro* models was performed to deeper investigate the role played by miR-223 in relation to the presence of HPV.

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.

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One of the hottest topics in the scientific research is to find new relevant molecular biomarkers to facilitate early specific diagnosis for rare pathologies. Gynocare Action strongly supports this goal and encourages the steps in this direction. The current STSM aimed to show the link between HPV and the potential biomarker miR-223 in HPV positive Laryngeal Cancer, which is a rare pathology in women.
The experimental work carried out during this STSM led to preliminary conclusions which are in line with the original hypothesis and with the deliverables of the mission. In details, the results show an upregulation of expression level of TG2 in HEp-2 overexpressing miR-223 if compared to its negative control. In contrast, a down regulation of TG2 was found in the HEp-2 inhibiting the mirR-223, if compared to the negative control and to HEp-2 overexpressing miR-223. Moreover, these different expression levels of TG2 were not emphasised in the HNO-210 cell lines, suggesting an interesting relation amongst miR-223 and the presence of HPV. Additionally, the modulation of TG2 protein in Hep-2 cell lines also corroborates with the expression of NFKB, which was significantly higher expressed, in both total and phosphorylated form in the HEp-2 overexpressing miR-223 than its negative control and HEp-2 inhibiting miR-223. These findings, that were in line with the described expectations such as “to compare protein targets in HPV-positive and negative in vitro models of LCa” or “to investigate the role played by the potential biomarker miR-223 in specific cellular pathways”, suggested an oncogenic role played by the miR-223 especially in those HPV positive cell lines. This prospect was then confirmed with the *in vitro* functional assays. Although an increased proliferation and tendency to migrate was detected in both LCa cell lines (Hep-2 and HNO-210 over expressing miR-223 cells) if compared to their negative controls, it was interesting to see how these effects were already evident in a shorter time for positive HPV cell lines than HPV negative ones. Therefore, the achieved results suggest that HPV positivity in LCa could turn into more aggressive tumour behaviour than negative ones through the regulation of relevant oncogenic proteins. These results appear in line with previously reported scientific evidence of increased TG2 expression in the HPV-positive premalignant cervix lesions. Furthermore, the outcomes reinforce the proposed hypothesis of the involvement of the oncogenic miR-223 in HPV-positive laryngeal cancer through the overexpression of proteins related to cancer progression. To exhaustively demonstrate this correlation a proteomic analysis was also performed. The results will reflect a broader vision than what has been studied so far. In conclusion, the STSM allowed to deepen the possible mechanism behind a rare pathology for women. Additionally, as a deliverable for gynocare mission, during the STSM it has been established a fruitful and ongoing collaboration between different professional researchers which had as objective the scientific progress in rare pathologies field and will culminate in publication of the findings.