ABSTRACT

Trefoil Factor 1 (TFF1) is a small secreted protein, belonging to the trefoil factor family, characterized by a conserved “trefoil domain” containing six cysteine residues that form a three loop disulphide structure. It is expressed in the gastrointestinal tract, where plays an essential role in mucosal protection through mucous-barrier formation, and also in mucosal repair through promotion of restitution after injury. In recent years clinical and experimental studies have shown an active function of the trefoil peptides in the genesis of neoplastic processes. TFF1 is mainly associated with breast cancer and gastric cancer (GC), but have been described changes in expression levels also in pancreatic cancer, lung, prostate and colorectal. TFF1 had been described as a tumor suppressor gene in gastric cancer, but it is markedly elevated in gastric mucosa with atypical hyperplasia, diffuse-type gastric cancer and with lymph node metastasis. However, the distinct signalling pathways have not been fully elucidated, nor have definitive functional receptors for trefoil proteins been identified.

In this PhD project, experiments were performed to understand the role of TFF1 in human GC development with particular attention to invasion and epithelial-mesenchymal transition (EMT) processes. Previously it has been demonstrated that TFF1 selectively binds copper ions, which influence homodimer formation and its biological activity. Here, by using TFF1 recombinant protein on AGS cell line and a TFF1 over-expressing clone (AGS-AC1), we demonstrated that TFF1 stimulated invasion of GC cell lines. The pro-invasive activity of TFF1 was strictly regulated by copper and was associated with a greater MMP-2 activity. We also reported that TFF1 was implicated in the occurrence of EMT, not only in the GC models but also in a prostate cancer cell line, in a same manner with a reduction of epithelial markers such as E-cadherin and cytokeratins 8 and 18 and an increase of mesenchymal ones such as vimentin. Additionally, in hypoxic condition, a significant increase of TFF1 expression was associated with hypoxia-related mesenchymal/metastatic process.

Furthermore, we demonstrated a TFF1 auto-induction mechanism with the identification of a specific responsive element located between -583 bp and -212 bp region of its promoter. This region is responsive to the presence of TFF1 and able to positively regulate its expression also during hypoxia and synergistically with HIF1-α induction. Additionally, we observed that TFF1 can regulate the methylation status of its promoter. We hypothesized that it can auto-activate its own expression regulating the density of methylated CpGs.

Finally, we investigated the relationship between TFF1 and the N-formyl peptide receptors (FPR1, FPR2 and FPR3), involved in innate immunity, inflammation and cancer, including GC. For the first time we reported a functional relationship between TFF1 and FPRs. In particular, we found that recombinant TFF1 protein in AGS cells induced FPR expression and FPRs influenced pro-invasive activity of TFF1.