and serum antibody titers were similar between severe and non-severe groups. However, we observed that individuals recovered from severe COVID-19 have a significantly reduced frequency of spike specific IgG + memory B cells expressing Tbet and FcRL5 (markers associated with long lived immunity). In the non-severe patients, we observed IgG +Tbet+ B cells targeting the spike protein peak at 2-3 weeks post-symptom onset, decrease by almost fifty percent 4-5 weeks post-symptom onset, and return to baseline 5 months post-symptom onset. Our study also validated previous findings of a short-lived primary response of IgM+ B cells targeting the spike protein. DISCUSSION/ SIGNIFICANCE: Our findings highlight potential implications for long-term immunity against re-infection or severity of the resulting disease in patients with severe COVID-19. Further investigation will be necessary to determine whether the maintenance of immunological protection is hindered in patients who overcame severe COVID-19.

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Post-translational role of RNA modifications in sRNA chaperone Hfq

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OBJECTIVES/GOALS: The goal of this study is to determine the role of the tRNA modifications in the translation of Hfq. Hfq is an RNA chaperone that acts as a co-factor for the action of the largest class of small RNAs in E. coli. RNA modifications have been known to play critical roles in the translational fidelity of many cellular proteins in bacteria. METHODS/STUDY POPULATION: In this study, we used an hfq-lacZ translation fusion to screen several RNA modification mutant genes to uncover additional RNA modifications that may play a role in Hfq translation. We measured hfq-lacZ activity in genetic backgrounds mutated for several additional RNA modification enzymes previously untested for Hfq effects. RESULTS/ANTICIPATED RESULTS: We identified 5 RNA modification genes that were defective for hfq-lacZ fusion activity, and we subsequently performed western blot analysis on the Hfq protein in the absence of these modification mutant genes to determine the effect of these mutants more directly on Hfq protein levels. We identified 2 out of these 5 RNA modification mutants that also affect Hfq protein levels. DISCUSSION/ SIGNIFICANCE: Since Hfq is critically important for small RNA function is a wide range of bacteria, it is possible tRNA modifications regulate Hfq expression in other bacteria. These processes, when further investigated, could provide us with the basic information to develop new antibiotics needed to address emerging antibiotic resistance.

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Inhibition of GPR30 Reveals Putative Genes Involved in the Pathogenesis of Inflammatory Breast Cancer

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OBJECTIVES/GOALS: Inflammatory Breast Cancer (IBC) is the most aggressive form of breast cancer and does not have targeted therapy. GPR30, a 7-transmembrane estrogen receptor, may play a role in regulating cell growth and proliferation of cancerous cells. Here, we evaluated changes in gene expression while inhibiting GPR30 to determine putative targets to treat IBC. METHODS/ STUDY POPULATION: IBC cell lines (SUM149PT) were cultured in medium with serum stripped from growth factor and hormones for 48 hours. Cells were then exposed to either G15 (GPR30 inhibitor) at a concentration of 1ÂµM or ETOH (vehicle negative control) 3 hours in triplicates. After exposure, total RNA was extracted using the Qiagen RNAeasy Mini kit and RNA was sequenced using the Illumina NextSeq (2 X 75bp). The higher-quality reads were aligned, annotated, and quantified to the human genome (HG38) using STAR and RSEM softwares. Gene expression analysis was performed in R statistical software (packages tximport and DESeq2). Functional and enrichment analyses were performed using Metascape and STRING database, respectively. RESULTS/ANTICIPATED RESULTS: There were 656 significantly expressed genes ($p < 0.05$) between groups (G15 vs. ETOH). The top 5 significant genes include: SMIM7, FANCG, ARID1A, MAML2, and ATF3. Significantly impacted biological processes and pathways include: electron transport chain, mitotic cell cycle process, microtubule cytoskeleton organization, cellular component morphogenesis and DNA-dependent DNA replication (adj $p < 0.05$). Additionally, physical and functional interaction networks showed 3 major clusters (â‰¥ 12 genes), which contained several gene hubs including BRCA1, BRCA2, FOS (proto-oncogene), PLK1 and PAK1 (both serine/threonine-protein kinases), among others. Interestingly, the network analysis showed the previously known interaction between FANCG and BRCA2, which were both dysregulated by GPR30 inhibition. DISCUSSION/SIGNIFICANCE: Through gene expression, functional and enrichment analyses we found several targets genes that could be associated with the pathogenesis of IBC. Validation of candidates genes (qRT-PCR and Western blot), and functional assays (cell proliferation, motility, and invasion) will be performed to understand the potential of these genes in treating IBC.

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Isolation and identification of bioprospects capable of metabolizing 17-beta-estradiol and 17-alpha-ethinylestradiol using metagenomics and culture-dependent techniques in Puerto Rico

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OBJECTIVES/GOALS: This research project aims to isolate and identify bioprospects capable of metabolizing estrogen using culture-dependent, culture independent methods and the identification of the gene/genes responsible for the metabolization of estrogen by the bioprospects. METHODS/STUDY POPULATION: For the culture dependent technique, samples were collected from the water treatment plant in MayagÃ¼ez, cultivated on TSA medium and selected specific and diverse colonies were patched on M-9NC (no carbon sources), M-9-glucose (M9G) and M-9-hormone mixture (M9H: 17-beta-Estradiol and 17-alpha-Ethynylestradiol). After the 48hrs incubation at 25 and 37 Celsius, growth was scored on the different media, to choose those potential bioprospects that use the hormones as the sole carbon source. For the culture independent approach, metagenomic clones from libraries generated from the