"Effects of HDAC6 inhibition on the functionality of the transcription factor STAT3 on colorectal cancer cells"

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ABSTRACT

Cancer is one of the most frequent disease worldwide and currently, treatment failures are caused by pharmacological resistance or low success rate of conventional approaches. In particular, colorectal cancer (CRC) has a high incidence and mortality rates, which is why there is a need to study new strategies for future therapies. Histone deacetylase 6 (HDAC6), a mainly cytoplasmic protein, is involved in several cellular processes including tumor immunogenicity. Specifically, it has been observed that HDAC6 stimulates STAT3 activity, a transcription factor involved in immunogenicity. STAT3 plays a role as a transcriptional inducer of different genes, as PD-L1 (programmed death ligand 1), while over-expression of PD-L1 on some cancer cells promotes inhibition of T lymphocyte activation in immune response. Therefore, we will focus on pharmacological inhibition of HDAC6 in CRC cell due to its potential as adjuvants to avoid immunotolerance in cancer therapy. Here, we investigate whether HDAC6 affects STAT3 activation in CRC cells. Treatments with Nexturastat A (NextA), a specific HDAC6 inhibitor, decreases the levels of activating post-translational modifications on STAT3 analyzed by Western blot and immunocytochemistry. These results suggest that treatments with specific HDAC6 inhibitors would reduce the functionality of STAT3 in CRC cells. Therefore, the use of specific HDAC6 inhibitors may be a reasonable and an interesting adjuvant strategy for immunotherapy, since HDAC6 inhibitors would indirectly decrease PD-L1 expression, one of the checkpoint inhibitors of immune system

INTRODUCTION

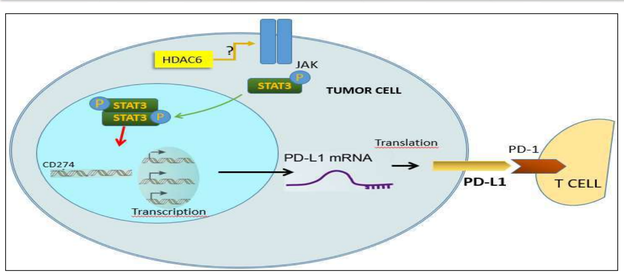


Fig 1. Representative scheme of PD-L1 transcriptional regulation by STAT3 transcription factor. HDAC6 is involved in STAT3 phosphorylation, then phosphorylated STAT3 homodimerize and translocate to the nucleus to activate transcription of its targets. Cytoplasmic HDAC6 can deacetylate or modulate members of the STAT3 activation pathways

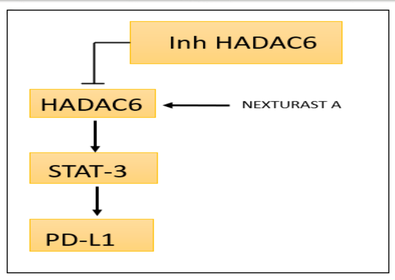
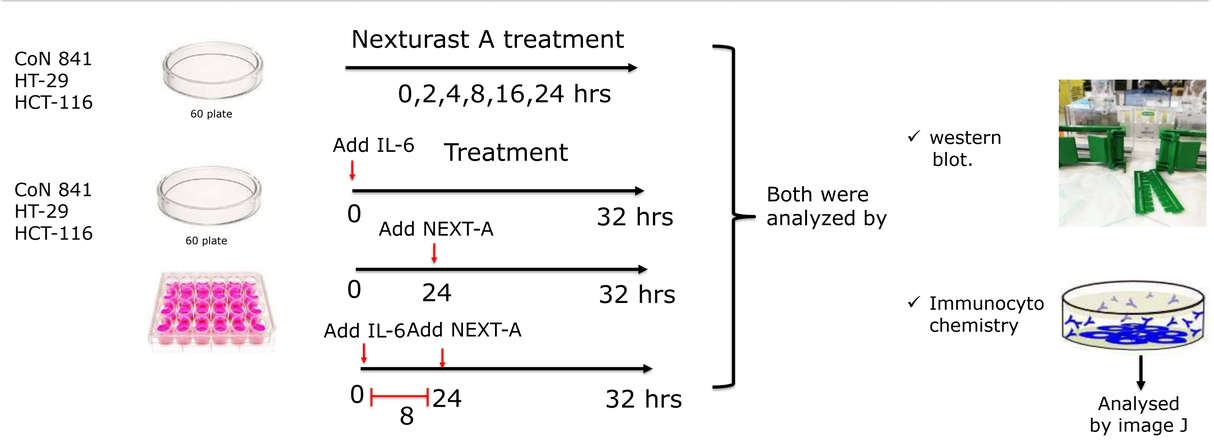
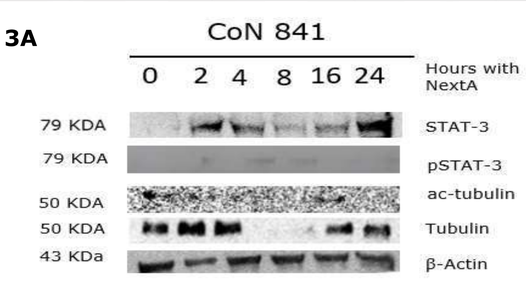


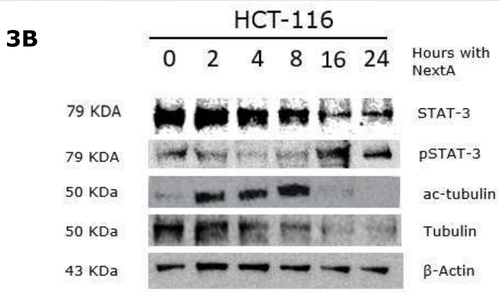
Fig 2. HDAC6 controls the activation of STAT3 and the expression of PD-L1. It has been reported that HDAC6 inhibitors indirectly modulate the expression of PD-L1 in other types of cancer through STAT3 activation, decreasing PD-L1 expression. This is unknown in colorectal cancer

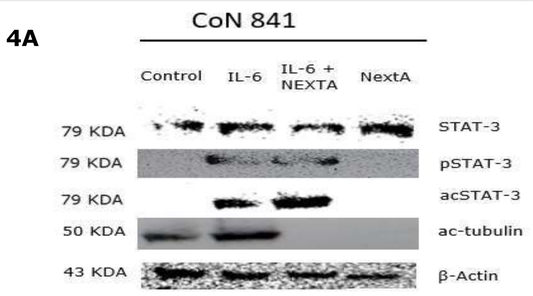
METHODS

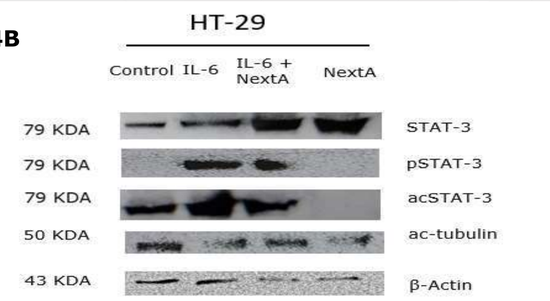


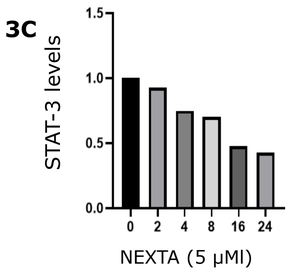
RESULTS

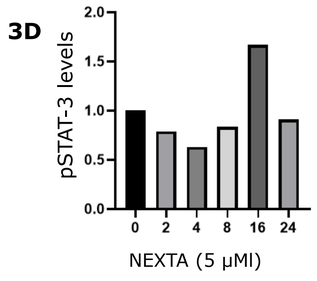












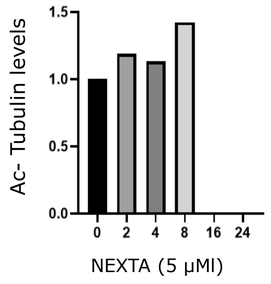
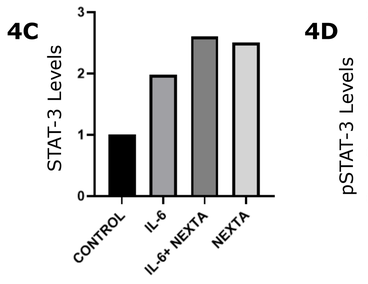
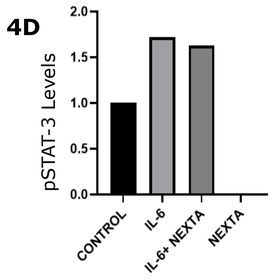
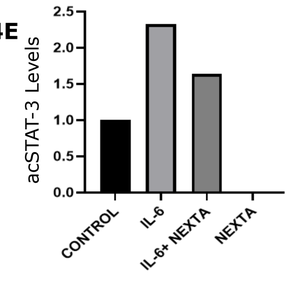


FIG. 3. STAT3 regulation through HDAC6 inhibition with NextA, A) CoN 841 cells were treated with NextA, for 24 hours, western blot analysis using specific antibodies was used. B) HCT 116 cells were treated with NextA, for 24 hours, western blot analysis using specific antibodies was used. C-D-E-F) Quantification of antibodies in HCT 116 cells (NextA was used at 5 uM, cell signaling antibodies).







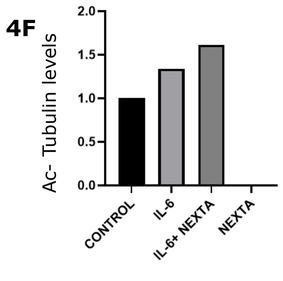


FIG. 4. STAT3 regulation is shown when HDAC6 is stimulated and inhibited via IL-6 and NextA, A) CoN 841 cells were treated with IL-6 and NextA, for 32 hours, western blot analysis using specific antibodies was used. B) HT 29 cells were treated with IL-6 and NextA, for 32 hours, western blot analysis using specific antibodies was used. C-D-E-F) Quantification of antibodies in HT-29 cells (IL-6 was used at 0.2 ug/ml and NextA was used at 5 uM, cell signals antibodies).

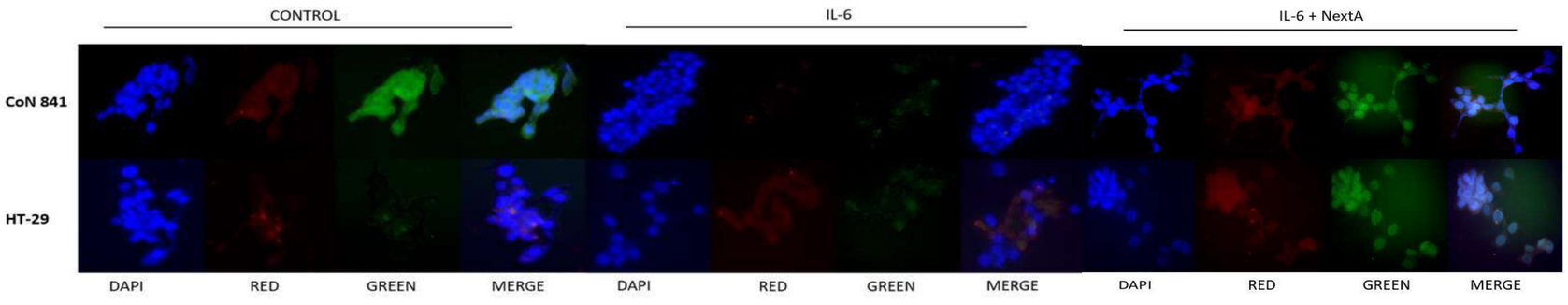


FIG. 5. co-localization of STAT3 and nucleus, IL-6 was used as a stimulator and NextA as an inhibitor, markers were DAPI, RED corresponding pSTAT3 and GREEN corresponding total STAT3, the antibodies that provided more information were traced in HT 29 cells, which were total and phosphorylated STAT3. (IL-6 was used at 0.2 ug/ml and NextA at 5 uM, the cell signals of the antibodies).

CONCLUSIONS

- The CRC cell lines used, express STAT3 and show both modifications (acetylation and phosphorylation), even more so when treated with IL-6 which promotes swelling generated by the tumor immune system.
- HDAC6 inhibitor (NextA) can modify STAT-3 expression by decreasing protein levels. We can suggest that HDACinh affects STAT-3 function in CRC cells. But more repetition are needed