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Title

TGF-beta induces ribosome activity, alters ribosome composition and inhibits IRES-mediated translation in chondrocytes

Purpose

Ribosomes are required for the continuous translation of all proteins in cells. In the last five years, it has become clear that this is not a static process, but subject to regulation. Recently, it was discovered in a high throughput screening that thousands of human mRNAs contain a 5' untranslated region that regulates protein translation through Internal Ribosomal Entry Sites (IRES). In addition to the major pathway of Cap-mediated translation, IRES-mediated translation is a unique level of regulation of protein translation that is active in many different conditions requiring specialized protein production. We used a candidate approach in a chondrocytic cell line to identify growth factors or cytokines that differentially regulate IRES-mediated translation. We hypothesized that external stimuli, relevant to cartilage homeostasis, alter ribosome function in chondrocytes with relevance to OA(OA) pathophysiology.

Methods

The SW1353 chondroytic cell line and human articular chondrocytes (HACs) from joint replacement surgery were cultured in DMEM/F12 with 10% FCS, NEAA and antibiotics. Cells were stimulated for up to three days with 12 different growth factors (TGFb, BMPs, WNTs, FGFw, IGF, etc.), cytokines (Interleukins, TNFa) and environmental stimuli (increased osmolarity) that are relevant for OA and joint homeostasis. SW1353 were transfected with validated bicistronic reporter constructs containing either a CrPv IGR, HCV or P53 IRES using Fugene. Total protein translation was evaluated by incorporation of ^{35}S-methionine/cysteine normalized to DNA content. Ribosomes with associated proteins were isolated from cytoplasmic extracts using a sucrose cushion and ultracentrifugation, which was evaluated by immunoblotting (GAPDH, H3) and RNA agarose gel electrophoresis (rRNA). Unbiased identification and quantification of the cellular proteome, purified ribosomes (with associated proteins) and secretome was performed using liquid chromatography tandem mass spectrometry and label-free quantification. Fold change and significance cut-offs forproteomic analyses were fold change > 2.0 and p-value < 0.05.

Results

Our initial IRES activity screening revealed that Transforming Growth Factor beta 3 (TGFβ3) consistently decreased IRES activity in favor of Cap-mediated translation by a factor of 1.5-2.0 fold. A concentration of ≥ 1 ng/ml TGFβ3 and at least two days of stimulation was required for this effect. Subsequently, we tested whether TGFβ3 increased total protein translation in SW1353. This was significantly increased by TGFb3 by 1.1 fold without affecting proliferation and this action could be inhibited with the ALK5/TGFβR1 inhibitor SB505124. TGFβ3 also increased protein translation in HACs by 1.5 fold with a moderate effect on proliferation. These results indicate a global inhibition

of IRES-mediated translation and a concomitant increase in Cap-dependent protein translation.

To identify what proteins were differentially expressed, we utilised mass-spectrometry label-free quantification. In the cellular proteome we found that TGFβ3 induced protein expression of known TGFβ transcriptional target genes at day 3 compared to control, such as JUNB (19.2x), FN1 (3.9x) and TGFβ2 (4.4x). This was also reflected in the secretome with significant up regulation of SERPINE1 (5.8x), FN1 (2.2 fold) and TGFβ2 (2.3 fold). Interestingly, ALPP (-5.0x) and ALPP2 (-20.1x) were repressed by TGFβ3 treatment in the cellular proteome and MMP3 (-5.6x) in the secretome.

Finally, we isolated ribosomes with their associated proteins from control and TGFβ3-treated cells. Ribosomes isolated from TGFβ3-treated cells contained significantly lower amounts of the Heterogeneous RiboNuclear Protein (HNRNP) family members A0 (6.6x), A1 (3.4x), A3 (3.9x), C (2.7x), H1 (2.2x), L (3.2x), M (2.6x), R (2.2x) and U (4.1x). The majority of these HNRNPs were not differentially expressed in the cellular proteome or only to a small degree (1.2x). Of note, significantly increased association of eukaryotic initiation factor 2 (A, S1, S2, S3; 1.3-1.4 fold) and six tRNA ligases (DARS, EPRS, IARS, KARS, RARS, QARS; 1.6-2.2x) were found on ribosomes isolated from TGFβ3-treated cells, supporting the observed increased polypeptide incorporation rate of 35S-methionine/cysteine following TGFb3 treatment. Finally, a small (1.1-1.2x) but significant increase in specific ribosomal proteins was found in ribosomes extracted from TGFβ3-treated cells (RPS4X, RPS8, RPL13a, RPS15, RPS21, RPS27A).

Conclusions

We demonstrate that a cytokine/growth factor can induce; ribosome activity,changes in ribosome composition and preferential mode of translation in eukaryotic cells.

The effect on preferential translation required at least 2-3 days and was not measurable at day 1. Furthermore, this effect was specific for TGFβ3 (≥1ng/ml), could be prevented by inhibiting TGFβR1 receptor kinase activity, and was not evident for other evaluated growth factors/cytokines. Ribosome proteomic analyses identified the HNRNP family as being less abundantly associated with ribosomes in TGFβ3-treated cells. HNRNP family members are known to function as IRES-transacting factors and their reduced ribosomal association after TGFβ treatment may be responsible for the observed decreased IRES-mediated translation.

TGFβ3 is crucial for cartilage homeostasis and OA pathology. This new level of regulation can be important for cartilage biology and the ribosome may be a drugable target in OA, as was shown in oncology research. Future experiments will focus on functional validation of the link between HNRNP down regulation and decreased IRES-mediated translation. Moreover, we have generated bicistronic reporter constructs with 5’ UTRs and IRESs of genes involved in OA that will be evaluated in future screening experiments.