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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 addition to the major pathway of Cap-mediated translation, Internal Ribosomal Entry Site (IRES) mediated translation is a distinct level of translational regulation that can be activated under stress conditions (Fig.1A). Recently, it was discovered that thousands of human mRNAs contain a 5’ untranslated region that regulates protein translation through IRESes. 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 osteoarthritis (OA) pathophysiology.

Methods

The SW1353 chondrocytic cell line and human articular chondrocytes (HACs) from knee 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 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 35S-methionine/cysteine and normalized to DNA content. Ribosomes with associated proteins were isolated from cytoplasmic extracts using a sucrose cushion and ultracentrifugation. 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.

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 (Fig. 1B). 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. TGFβ3 significantly increased this by 1.1 fold without affecting proliferation (Fig.1C). 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 utilized 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 TGFB2 (4.4x). This was also reflected in the secretome with significant up regulation of SERPINE1 (5.8x), FN1 (2.2 fold) and TGFB2 (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, Fig.1D). 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 incorporation rate of 35S-methionine/cysteine. Finally, a small (1.1-1.2x) but significant increase of specific ribosomal proteins was found in ribosomes extracted from TGFβ3- treated cells (RPS4X, RPS8, RPL13a, RPS15, RPS21, RPS27A).

Conclusions

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

The effect on preferential Cap translation required at least 2-3 days and was specific for TGFβ3 (≥1ng/ml). 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 TGFβ3 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 ribosome-associated HNRNP abundance and alterations in IRES-mediated translation in chondrocytes.