Human *Argonaute 1* 5' untranslated region can mediate cap-independent translation initiation *via* an internal ribosome entry site

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Argonaute proteins (AGOs) are essential effectors in RNA-mediated gene silencing pathways. There are eight AGO-like proteins in human cells, grouped in two families: the eIF2C/AGO subfamily and the PIWI subfamily. The eIF2C1 gene encodes AGO1, a member of the former subfamily, that is ubiquitously expressed at low to medium levels and it is highly conserved during evolution reflecting its important physiological roles. Moreover, recent studies concluded that AGO1 protein is overexpressed in colorectal cancer, relative to adjacent non-cancer tissue, without a concomitant increase in mRNA levels. These pieces of evidence lead us to suspect that high AGO1 protein levels may be due to internal ribosome entry site (IRES)-mediated translation. IRESs are structures that can mediate cap-independent translation initiation by directly recruiting ribosomes to the AUG vicinity, thus skipping the scanning of the whole 5' untranslated region (UTR), in response to stress. To confirm this hypothesis, we transiently transfected colorectal cancer HCT116 and cervical cancer HeLa cells with an AGO1 5'UTR-containing dicistronic vector, and luciferase activity was measured by luminometry assays. Results have shown a 2-fold increase in relative luciferase activity in both cell lines, when compared to the cells transfected with the empty counterpart (P<0.05). Transfection of the corresponding promoterless plasmids ruled out the hypothesis of this fold to be due to the existence of a cryptic promoter. In addition, RT-PCR analyses of the dicistronic mRNAs confirmed that no cryptic splicing occurs. Besides, the knock-down of the eIF4E subunit induced a significant 2- to 4-fold increase in IRES activity. Taken together, these data suggest the presence of an IRES in the AGO1 5'UTR whose biological relevance is still under thorough investigation.