MKK3 TARGETING AS A PROMISING THERAPEUTIC TOOL IN COLORECTAL CARCINOMA

68

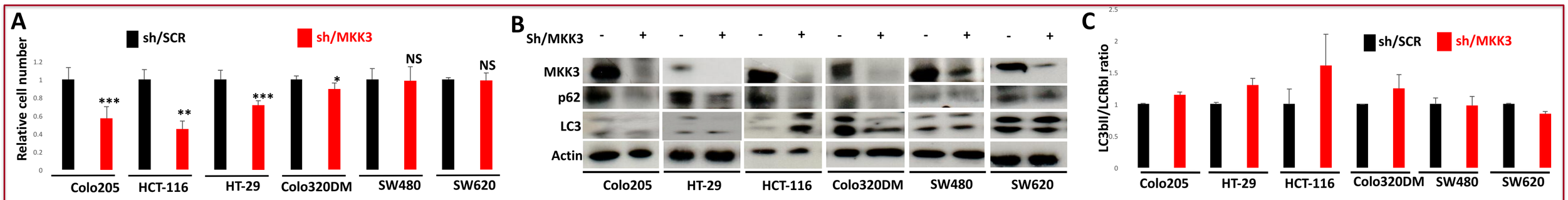
Stramucci L[1], Pranteda A[1], Amoreo CA[2], Diodioro MG[2], Bossi G[1]

[1]Laboratory of Medical Physics and Expert Systems and [2]Department of Pathology, IRCSS "Regina Elena" National Cancer Institute, Rome

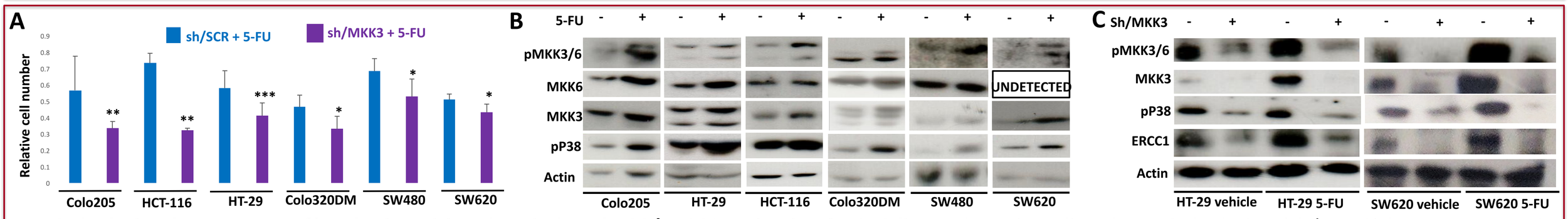
BACKGROUND AND RATIONALE

We have previously reported that MKK3 targeting results into induction of autophagic cell death and in vitro and in vivo boosting of 5-Fluorouracyl (5-FU) antitumor efficacy in breast cancer and colorectal carcinoma (CRC) cell-lines. CRC is a highly aggressive disease and patients urgently need the development of targeted therapies in order to improve prognosis: here we characterize the effects of MKK3 silencing in CRC in order to explore MKK3 suitability as a perspective therapeutic target.

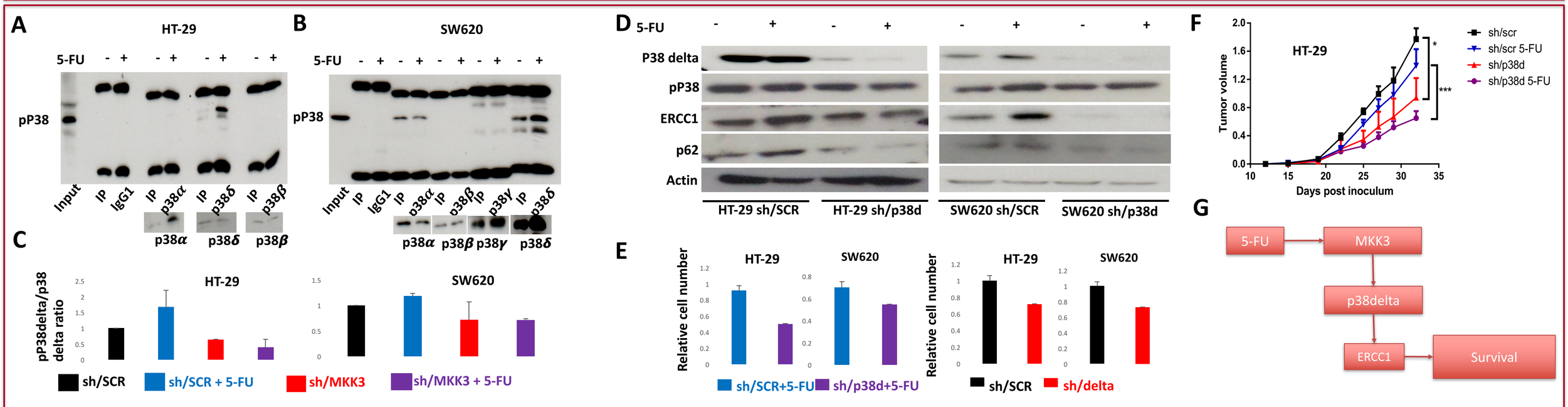
RESULTS



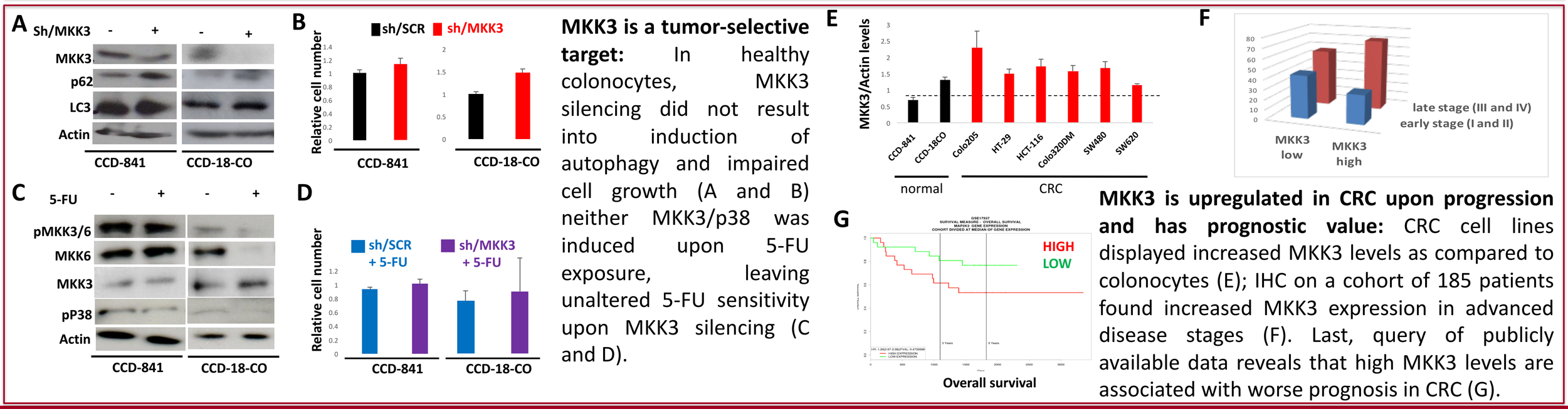
MKK3 depletion impairs cell growth in several CRC lines by inducing sustained autophagy: shRNAi-mediated MKK3 depletion (sh/MKK3) impairs cell growth in several CRC cell lines (A). shMKK3 cell lines showing impaired growth also display induction of autophagy as assessed by decreased p62 levels (B) and increased LC3II/LC3I ratio (B and C).



MKK3 depletion boosts 5-FU effect by impairing drug-induced p38/ERCC1 activation in CRC lines: shRNAi-mediated MKK3 depletion (sh/MKK3) boosts 5-FU anti-tumor effect in CRC cell lines (A). 5-FU induces MKK3/p38 signaling (B) resulting into ERCC1 stabilization (C). Targeting MKK3 shuts down p38/ERCC1 signaling avoiding its stabilization upon 5-FU exposure (C).



5-FU prosurvival signaling is mediated by MKK3/p38delta activation in CRC lines: p38 isoform-specific IP of 5-FU treated/untreated cell lysates revealed selective phosphorylation of p38 delta isoform (A and B) upon 5-FU exposure. Moreover, MKK3 silencing blocked 5-FU-triggered p38delta phosphorylation (C). Also, p38 delta silencing by stealth RNAi prevented 5-FU- induced p38/ERCC1 activation (D) boosting 5-FU effects (E), and induced autophagy impairing cell growth (D and E). Such effects were also observed in vivo (F), consistent with 5-FU mediating a MKK3/p38delta prosurvival signaling (G).



MKK3 is a tumor-selective target: In healthy colonocytes, MKK3 silencing did not result into induction of autophagy and impaired cell growth (A and B) neither MKK3/p38 was induced upon 5-FU exposure, leaving unaltered 5-FU sensitivity upon MKK3 silencing (C and D).

MKK3 is upregulated in CRC upon progression and has prognostic value: CRC cell lines displayed increased MKK3 levels as compared to colonocytes (E); IHC on a cohort of 185 patients found increased MKK3 expression in advanced disease stages (F). Last, query of publicly available data reveals that high MKK3 levels are associated with worse prognosis in CRC (G).

CONCLUSIONS

MKK3 represents an attractive therapeutic tool in CRC: MKK3 targeting is able to impair cell growth by inducing autophagy in different CRC cell lines in vitro, and boosts 5-FU effect in vitro in all of the CRC cell lines tested by blocking 5-FU-induced prosurvival signaling mediated by p38delta/ERCC1 activation. Consistent with our previous report of MKK3 silencing being able to impair tumor growth and boost 5-FU effects in vivo, we were able to observe the same phenomenon by p38delta targeting. Moreover MKK3 targeting effects are tumor-selective and late stage CRC patients, displaying high MKK3 levels, would benefit from a perspective MKK3 targeting therapy.

Funded by the Italian Association for Cancer Research (AIRC): IG2016 Id:18449

IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA

15th Nov 2018

ISAB