

Prostaglandin E₂ Induces Human Enhancer of Filamentation 1 to Promote Spreading of Colorectal Carcinoma Cells

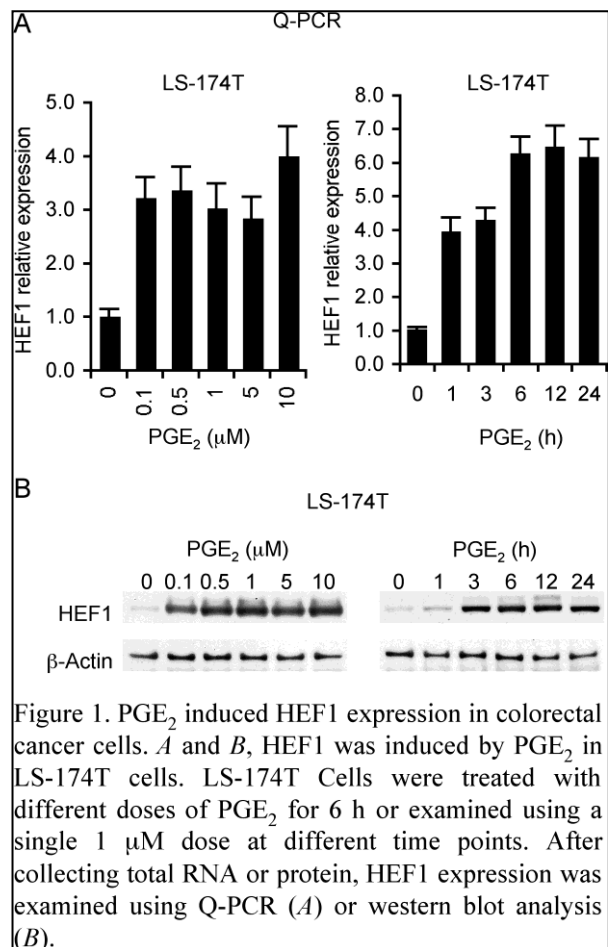
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Elevated expression of cyclooxygenase-2 (COX-2) and concomitant overproduction of Prostaglandin E₂ (PGE₂) have been directly linked to colorectal carcinogenesis. COX-2 inhibitors can suppress colorectal carcinogenesis, but long-term use can cause adverse effects in a subset of patients (1). Our research has focused on identifying and characterizing the effector molecules downstream of PGE₂-driven signaling involved in colorectal carcinogenesis. The ultimate goal is to develop approaches that have the same benefit, but result in fewer side effects. PGE₂ is the primary mediator of COX-2 mediated promoting cancer progression. Many studies have shown that PGE₂ promotes metastasis of colorectal cancer. The effects of PGE₂ on metastasis have been proposed to be through the activation of EGFR or upregulation of matrix metalloproteinases-2 and 9. Obviously, more work is needed to reveal the details by which PGE₂ signaling affects metastasis.

Recently, human enhancer of filamentation 1 (HEF1; also called NEDD9 or Cas-L) has been shown to promote metastasis of melanoma (2, 3). HEF1 is a scaffold protein that encodes multiple protein interaction domains. It has been implicated in numerous biological activities including metastasis.

Since both PGE₂ and HEF1 have been implicated in promoting metastasis in certain contexts, and HEF1 is regulated by PGE₂, we sought to better understand its role in colorectal cancer. Based on our initial findings, we hypothesized that PGE₂ induces HEF1 expression to



promote metastasis of colorectal cancers.

To test our hypothesis, we first examined the induction of HEF1 by PGE₂ using a colorectal cell line LS-174T. As shown in Fig. 1, PGE₂ rapidly stimulated the expression of HEF1 mRNA and protein (Fig. 1). HEF1 expression was also induced by PGE₂ in some other colorectal cancer cell lines, especially those with low basal HEF1 expression levels (data not shown).

On culture dishes, LS-174T cells tend to grow in a clump. PGE₂ treatment increased the numbers of lamellipodia and lipid vesicles and enhanced cell spreading as reported previously (Fig. 2A)(4). To examine whether HEF1 plays a role in PGE₂ induced cell spreading, HEF1 protein was overexpressed in stably transfected LS-174T cells (HEF1) and then compared to GFP control vector expressing cells (GFP) (data not shown). Stable HEF1 overexpression in LS-174T/HEF1 cells increased cell spreading

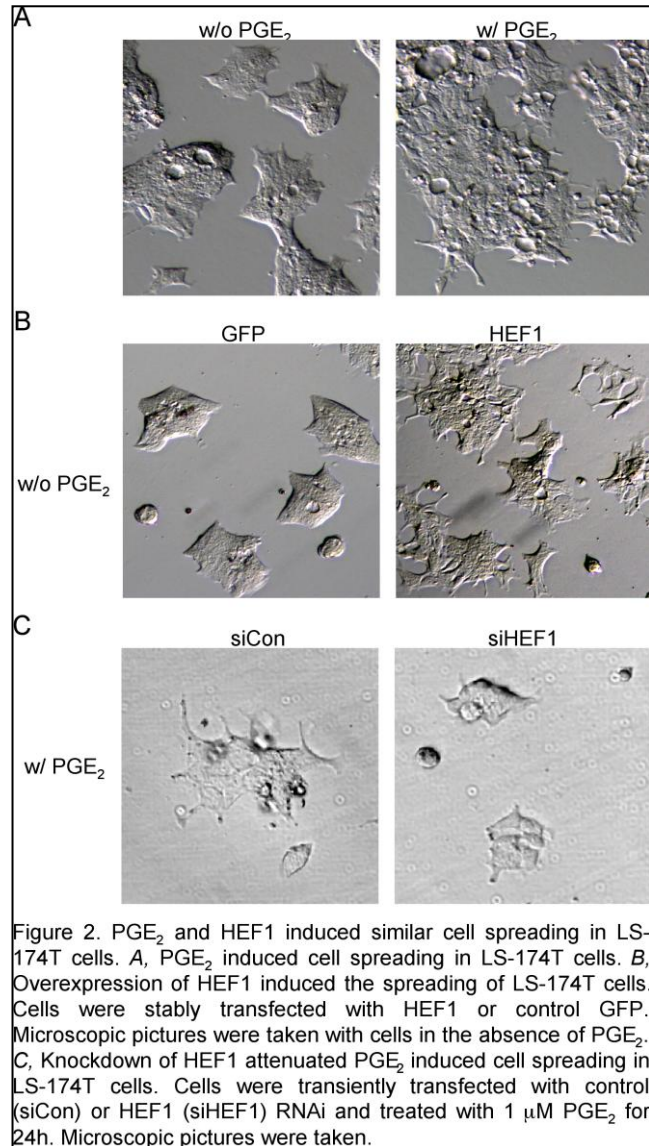


Figure 2. PGE₂ and HEF1 induced similar cell spreading in LS-174T cells. A, PGE₂ induced cell spreading in LS-174T cells. B, Overexpression of HEF1 induced the spreading of LS-174T cells. Cells were stably transfected with HEF1 or control GFP. Microscopic pictures were taken with cells in the absence of PGE₂. C, Knockdown of HEF1 attenuated PGE₂ induced cell spreading in LS-174T cells. Cells were transiently transfected with control (siCon) or HEF1 (siHEF1) RNAi and treated with 1 μM PGE₂ for 24h. Microscopic pictures were taken.

compared to GFP alone in LS-174T/GFP cells without treatment of PGE₂ (Fig. 2B). To further demonstrate that HEF1 mediates the effect of PGE₂ on cell spreading, HEF1 expression was knocked down using siRNA and then cells were treated with PGE₂. HEF1 expression was decreased to about 20% by a HEF1 siRNA compared to a non-silencing control siRNA (data not shown). Although the knockdown did not completely eliminate the expression of HEF1 protein, cell spreading was significantly reduced (Fig. 2C). These data suggest that increased HEF1 expression alone caused increases in cell spreading similar to PGE₂ treatment of parental LS-174T. Furthermore, spreading of LS174T cells in response to PGE₂ relies on HEF1 expression.

To elucidate the pathway involved in the induction of HEF1 by PGE₂, LS-174T cells were treated with Forskolin, a PKA activator. Forskolin induced HEF1 expression (Fig. 3A). In contrast, H-89, a PKA inhibitor, blocked the induction of HEF1 by PGE₂ (Fig. 3B). Knockdown of PKA by siRNA decreased HEF1 expression and attenuated the effect of PGE₂ on cell spreading (Fig. 3C and 3D). These data suggest that HEF1 induction by PGE₂ in LS-174T cells is through a PKA involved pathway.

In conclusion, we provide evidence that HEF1 is induced by PGE₂ and mediates its effects on promoting cell spreading. We are currently employing a colon orthotopic mouse model to validate whether our *in vitro* observations would translate into similar effects on metastasis. Together, these studies will add to our understanding of how PGE₂ regulates cell spreading and metastasis. The eventual goal is to help identify the possible targets downstream of COX-2 for the prevention and treatment of colorectal cancer.

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