The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

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7. SUMMARY AND CONCLUSION

Understanding the processes involved in cancer progression is of crucial importance for developing new prognostic markers and therapeutic targets for the treatment of cancer. OPN is a chemokine like protein, which is overexpressed by various types of cancer cells. Earlier reports have shown that OPN exerts the oncogenic and metastatic potential to the cancer cells (6, 7). OPN augments the expression of various genes involved in tumor growth, metastasis and angiogenesis whereas abrogates the expression or activation of genes/molecules involved in programmed cell death and protects the cancer cells from undergoing apoptosis.

We have shown the in-depth molecular mechanism involved in OPN-regulated prostate tumor growth, metastasis and angiogenesis. This study has provided insights into the crucial role of OPN in regulation of NF-κB-dependent COX-2 expression and PGE2-mediated prostate cancer progression via autocrine and paracrine mechanisms. We have demonstrated how COX-2 derived PGE2 regulates prostate tumor cell motility and angiogenesis via interacting with its receptors, EP2 and EP4. Consequently, the integration of information about the contribution of OPN and PGE2 in prostate tumor growth, metastasis and angiogenesis may illuminate the cellular changes at the molecular basis and it will be helpful in identification of novel molecular therapeutic targets and can clarify the potential utility of targeting OPN and COX2/PGE2 in different phases of cancer progression.

The following aspects have been addressed in this study

[5] The correlation between the expression profile of OPN with COX-2, NF-κB and the degree of angiogenesis in different grades of prostate cancer specimens.
The role of AP-1 and ATF-4 in PGE$_2$-regulated prostate tumor cell motility, uPA and VEGF expression.

The effect of PGE$_2$ on tumor angiogenesis and the role of EP2 and EP4 receptors in this process.

The correlation between the expression profile of EP2 and EP4 with AP-1, ATF-4, uPA and VEGF in various grades of prostate cancer.

The major findings of this study are as follows.

- The study demonstrated that purified human OPN augments COX-2 expression both at transcriptional and translational levels in human prostate cancer cells.
- OPN induces the COX-2 promoter activity via NIK and IKK mediated pathway.
- OPN regulates the PKC$_\alpha$ dependent c-Src activation.
- OPN induces the NIK dependent serine phosphorylation and NIK independent tyrosine phosphorylation of IKK$_\alpha/$β.
- Staurosporine (inhibitor of PKC) and dn-Src suppress the OPN-induced p65, NF-κB phosphorylation.
- Staurosporine, dn-Src and celecoxib (COX-2 inhibitor) inhibit OPN-induced PGE$_2$ production and MMP-2 activation.

Taken together, these data indicated that OPN regulates PKC$_\alpha$-c-Src dependent NIK-IKK-mediated NF-κB activation, which eventually regulates the COX-2 expression, PGE$_2$ production, MMP-2 activation in prostate cancer cells.

- Staurosporine, dn c-Src, celecoxib and EP2 blocking antibody suppressed OPN-induced prostate tumor cell motility and invasion.
- Blocking the PGE$_2$ receptor, EP2 on endothelial cells suppresses the OPN-induced tumor (PC-3)-derived PGE$_2$-mediated endothelial cell motility and invasion.

These results demonstrated that OPN regulated PKC$_\alpha$-c-Src dependent COX-2/PGE$_2$ mediated prostate tumor cell motility and invasion in EP2 receptor dependent autocrine manner. Moreover, OPN-induced tumor cell-derived PGE$_2$ induces endothelial cell motility and invasion via EP2 receptor dependent paracrine manner.
EP2 receptor blocking and celecoxib suppressed the OPN-induced xenograft tumor growth in nude mice.

Human prostate clinical specimen analysis showed the correlation between the expression of OPN with COX-2, NF-κB and neovascularization (vWF) in various grades of prostate cancer specimens.

These data showed that OPN induces the xenograft tumor growth in nude mice and inhibition of COX-2 and blocking the interaction of PGE\(_2\) with its receptor EP2 suppress OPN-induced PGE\(_2\)-mediated prostate tumor growth. Moreover, our in vitro and in vivo data further correlated with human prostate clinical specimen analysis suggesting that OPN can be used as a prognostic marker for prostate cancer progression.

PGE\(_2\) stimulates the phosphorylation of EGFR and β\(_3\) integrin in PC-3, prostate cancer cells. Moreover, PGE\(_2\) induces the expression of β\(_3\) integrin in PC-3 cells.

EP2 and EP4 receptor antagonists suppress the PGE\(_2\)-induced phosphorylation of EGFR and β\(_3\) integrin and expression of β\(_3\) integrin.

These data showed that PGE\(_2\) stimulates the activation of EGFR and induces the activation and expression of β\(_3\) integrin via EP2 and EP4 receptor.

PGE\(_2\) induces the expression of c-Fos and phosphorylation of c-Jun. It also induces the nuclear localization and DNA-binding of AP-1 and ATF-4 in prostate cancer cells.

Inhibition of EGFR and MEK or silencing β\(_3\) integrin suppressed PGE\(_2\)-induced AP-1 activation.

The overexpression of wt ATF-4 enhances but dn ATF-4 suppresses PGE\(_2\)-induced c-Fos expression, c-Jun phosphorylation and AP-1 DNA binding.

These results showed that PGE\(_2\) promotes EGFR and β\(_3\) integrin mediated AP-1 activation. PGE\(_2\) also regulates the crosstalk between ATF-4 and AP-1, which is unidirectional towards AP-1.
PGE₂ induces the expression of uPA and VEGF at translational and transcriptional levels.

Silencing EP2 and EP4 receptors curb PGE₂-induced uPA and VEGF expression.

Overexpression of dn ATF-4, dn c-Jun and A Fos suppress PGE₂-induced uPA and VEGF expression.

EP2 or EP4 receptor antagonist, overexpression of dn ATF-4, dn c-Jun and A Fos inhibit the PGE₂-induced PC-3 cells motility.

These data suggested that PGE₂ induces uPA and VEGF expression in prostate cancer cells via EP2 or EP4 receptor dependent AP-1 and ATF-4 mediated pathway. The EP2, EP4, ATF-4 and AP-1 play crucial role in PGE₂-induced PC-3 cell motility.

- PGE₂ stimulates the Akt and NF-κB, p65 phosphorylation in endothelial cells (HUVEC).
- AH6809 or AH23848 inhibit PGE₂-induced Akt, and NF-κB, p65 phosphorylation in endothelial cells.
- AH6809 or AH23848 suppress PGE₂-induced endothelial cell motility.
- Overexpression of COX-2 induces whereas silencing of COX-2 suppresses endothelial cell motility towards PC-3 cells. Moreover, the EP2 or EP4 receptor antagonists suppressed the PC-3 cell induced endothelial cell migration.
- The EP2 or EP4 receptor antagonists abrogated PGE₂-induced in vivo angiogenesis.
- The elevated expressions of EP2 and EP4 receptors were observed in higher grades of prostate cancer specimens as compared to the PIN and normal specimens, which further correlated, with expression profiles of ATF-4, c-Fos, c-Jun, uPA and VEGF.

The data coupled together suggested that PGE₂ induces tumor angiogenesis via EP2 and EP4 receptor dependent Akt and NF-κB mediated pathway. Our in vitro and in vivo data further correlated with human prostate cancer specimen analysis indicating the prognostic significance of PGE₂ and its receptors in prostate cancer.