Interactions of KLF4 and SIP1 in the regulation of E-Cadherin expression

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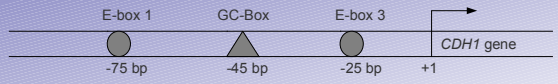
Background : **E-cadherin** down-regulation, a frequent event during carcinogenesis, is linked with poor prognosis in breast cancer patients. **KLF4** functions as an oncogene or tumor suppressor in a context-dependent manner. Recent studies show that KLF4 inhibits tumor progression and maintains E-cadherin expression by binding to its promoter. **SIP1**, a zinc-finger protein, binds two E-boxes of the E-cadherin promoter and represses its transcription. How SIP1 represses transcription is not clearly known. It has been suggested that SIP1, bound to two E-boxes, locks a promoter which is then less accessible for activating factors.

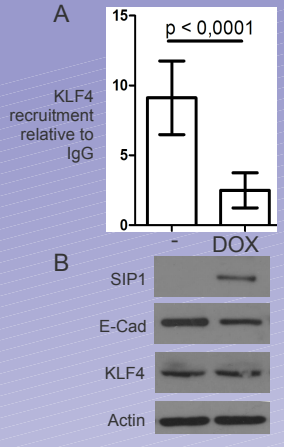
The E-Cadherin promoter



SIP1 is recruited by E-box -1 and -3. A GC-box, putative site for KLF4 binding is located between the two E-boxes.

SIP1 impairs the binding of KLF4 on the E-Cadherin promoter ...

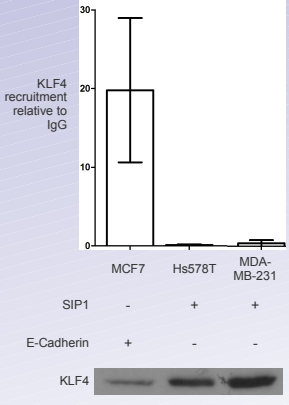
... in SIP1-inducible A431 cells



We generated A431 cells stably transfected with a vector allowing for the expression of SIP1 after treatment with doxycycline (DOX)
48hours after DOX was added, a Chromatin Immunoprecipitation assay was used to test for the binding of KLF4 (A).
Protein levels were checked by western blot (B)

Binding of KLF4 on the E-Cadherin promoter drops by ~3
While KLF4 is still expressed

... in three breast cancer cell lines

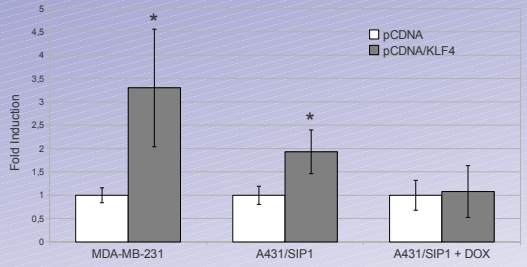


We also performed ChIP assays with cells expressing SIP1 and a cell line not expressing SIP1.
All these cell lines express KLF4.

In SIP1 expressing cells, KLF4 is absent from the E-Cadherin promoter

KLF4 and SIP1 have opposing functions on the -108/+19 region of the E-cadherin promoter

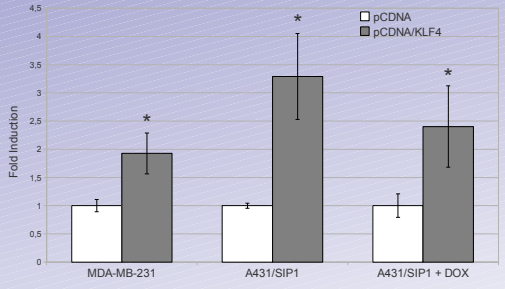
Cells were transfected with a reporter vector containing the -108/+19 region of the E-Cadherin promoter and a KLF4 expression vector. Luciferase activity was measured 48 hours after transfection



KLF4 activates the -108/+19 region.
SIP1 inhibits this activation («A431 + DOX» condition)
In MDA-MB-231 cells, overexpressed KLF4 can activate the promoter activity
→ Each factor can infere with the other

KLF4 binds to and activates the -66/-10 region of the E-cadherin promoter

By EMSA, we were able to locate more precisely a binding region for KLF4 (data not shown). This 56 bp region of E-Cadherin promoter is of particular interest, because it contains a GC-box surrounded by the two SIP1 binding sites.
We performed a luciferase assay with that -66/-10 region, which contains the GC-box and only one Ebox, which makes SIP1 unable to repress E-Cadherin expression.



When SIP1 binding is impossible, KLF4 transactivates the region, even if SIP1 is overexpressed

Work supported by : Fonds de la Recherche Scientifique, Centre anticancéreux près l'Université de Liège and Télévie

Conclusion : our results support the hypothesis that SIP1 exerts its repressive function on the E-Cadherin promoter by moving KLF4 away from it