# OSTEONECTIN (SPARC) EXPRESSION IN VASCULAR CALCIFICATION: *IN VITRO* AND *EX VIVO* STUDIES

**Paola Ciceri[1], Francesca Elli[1], Laura Cappelletti[1], Delfina Tosi[2], Federica Savi[2], Gaetano Bulfamante[2] and Mario Cozzolino[1]**

[1] *Laboratory of Experimental Nephrology and* [2] *Unit of Human Pathology, Departement of Helath Sciences, University of Milan, Italy*

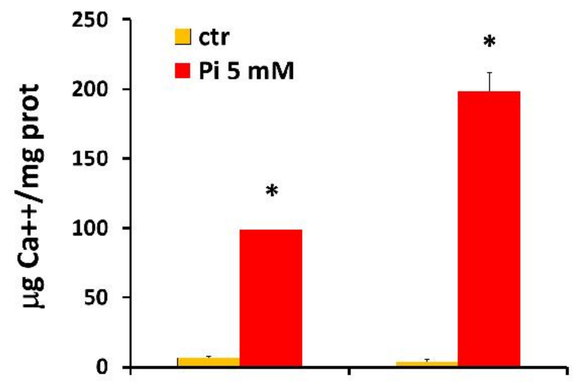
## Background and Aim

- **SPARC (secreted protein, acidic and rich in cysteine) is a non collagenous protein of bone matrix involved in cell differentiation, tissue remodeling and morphogenesis, secreted by fibroblasts, endothelial cells and vascular smooth muscle cells (VSMCs)**
- **VSMCs challenged with high phosphate (Pi) undergo to an active transformation in osteoblastic-like cells and mineralize their extracellular matrix: this process is called vascular calcification (VC)**
- **SPARC expression is modulated in calcific conditions: *in vitro*, it inhibits hydroxyapatite crystals formation, whereas SPARC knock-out prevents arterial calcification**
- **Since the role of SPARC in VC is not well elucidated, we tried to clarify its potential contribution *in vitro* and *ex vivo* studies**

## Materials & Methods

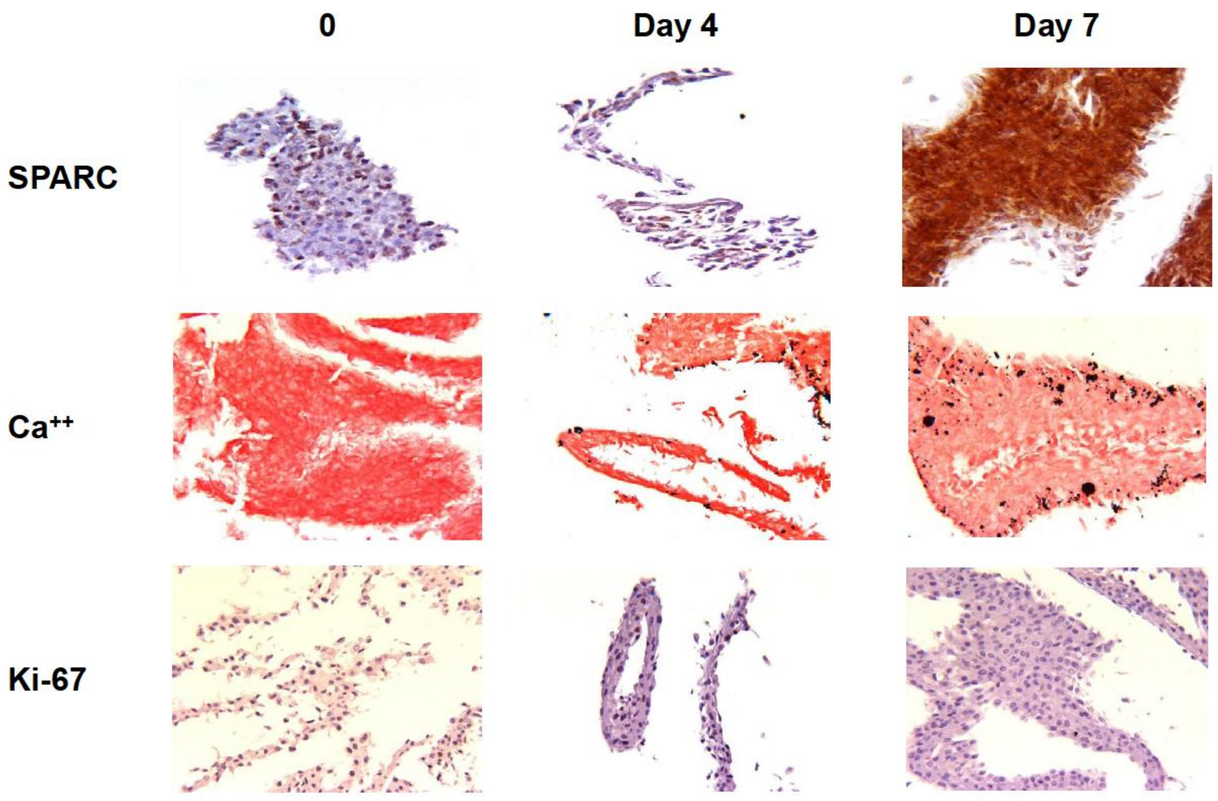
- **Rat VSMCs were cultured and challenged with inorganic phosphate (5 mM Pi) to induce calcification. (Calcification medium: DMEM high glucose,12% FBS, 10 mM sodium pyruvate, 100 U $ml^{-1}$ penicillin and 0.1 mg $ml^{-1}$ streptomycin and 50 ug/ml AA). Human arteries were isolated from adult with and without macroscopically evident atherosclerotic plaques**
- **Calcium (Ca) deposition was quantified by colorimetrical method and evaluated by histological analysis (Von Kossa staining)**
- **SPARC and Ki-67 protein expression was analyzed by immunohistochemistry**
- **Total RNA was extracted from rat VSMCs and Core Binding Factor alpha-1 (Cbfα-1/RUNX2) mRNA expression was evaluated by TaqMan PCR using β-actin housekeeping gene.**

## Pi-induced Calcium Deposition in VSMCs Is Time-Dependent

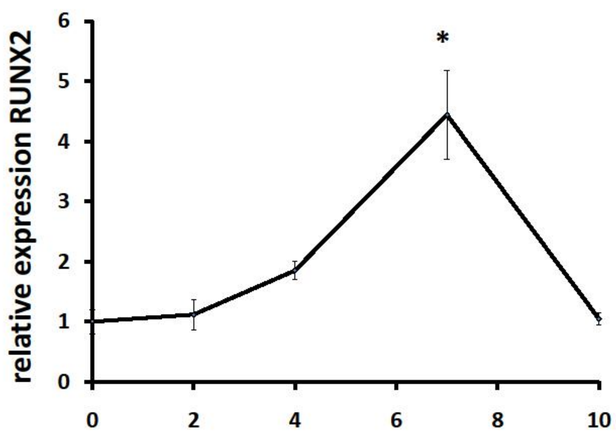



*p<0.01 ctr *vs* Pi

## SPARC Expression and Calcium Deposition Peak 7 Days After Pi Challenge, Whereas Proliferation Has a Different Time-course

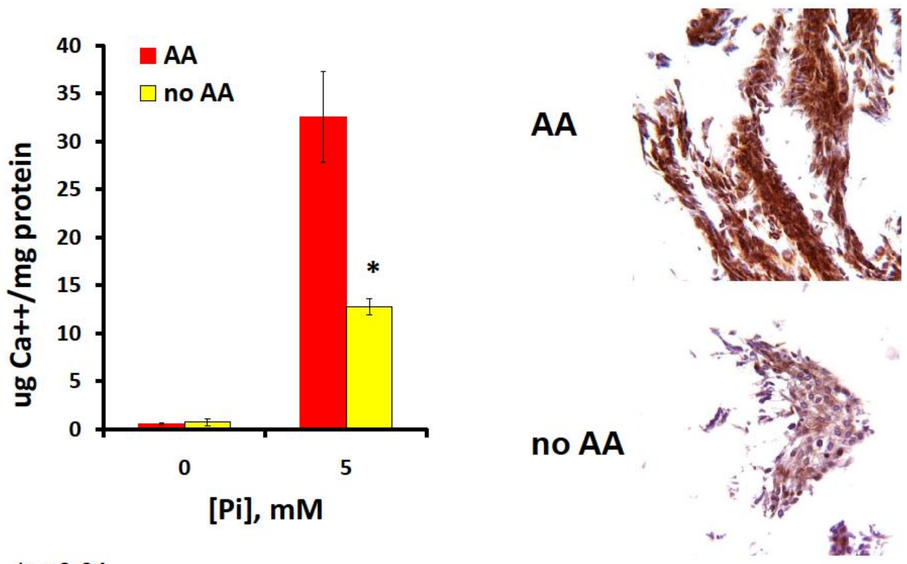



## RUNX2 mRNA Expression Peaks 7 Days after Pi Challenge



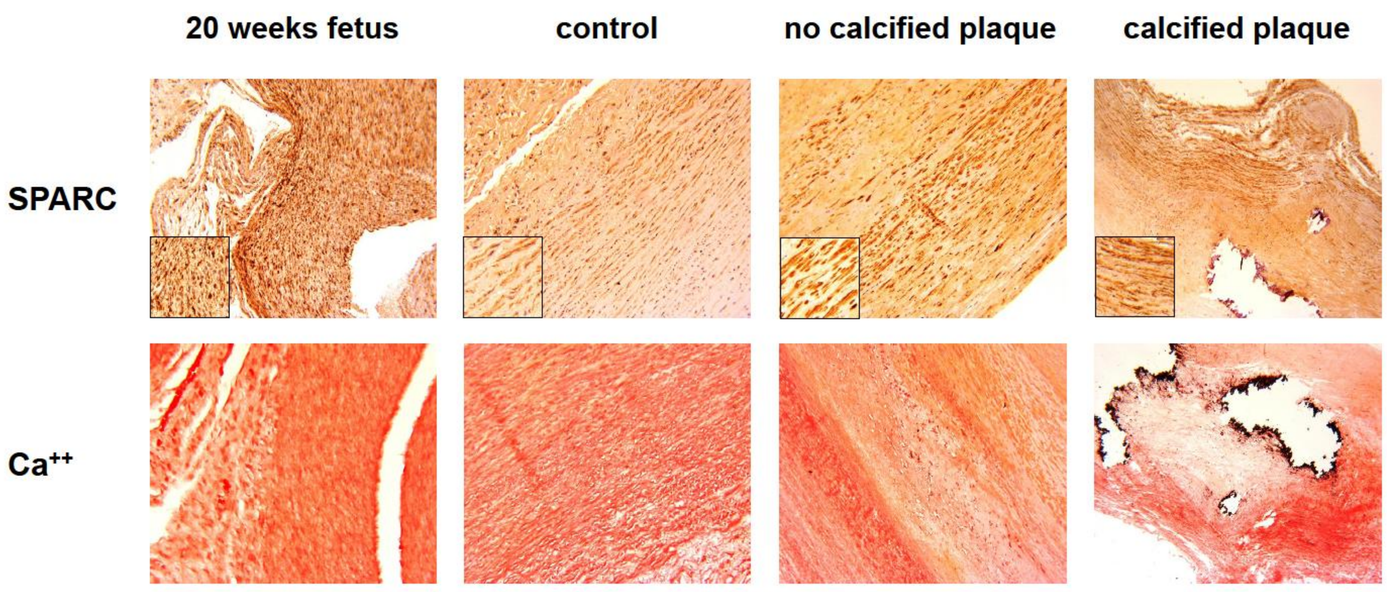

*p<0.01

## SPARC is Downregulated in Absence of the Pro-calcifing Factor Ascorbic Acid




*p<0.01

## SPARC Expression Increases in Human Arteries in Proximity of Site of Calcification




## Conclusions

**Our *in vitro* studies suggest that SPARC could have a potential pro-calcifying role in VC since its expression increases concomitantly with the massive Ca deposition and osteoblastic differentiation.**

**Moreover, SPARC *in vitro* expression is down-regulated in the absence of the pro-calcifying factor ascorbic acid.**

**Our *ex vivo* studies demonstrate that, with the progression of atherosclerosis, SPARC expression is up-regulated in the residual VSMCs at sites of arterial calcification, validating the hypothesis that SPARC actively partecipates to the calcification process.**

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H) Clinical Nephrology, CKD. H4) Bone disease in CKD 1-5.

Mario Cozzolino

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