ISSN 0025-7680

**SYMPOSIUM**

MEDICINA (Buenos Aires) 1999; 59: 553

# SUPPRESSION OF TUMOR CELL GROWTH BY TYPE IV COLLAGEN AND A PEPTIDE FROM THE NC1 DOMAIN OF THE α3(IV) CHAIN

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Our current studies are an outgrowth of earlier observations from our laboratory which have shown that basement membrane collagen (Type IV) inhibits activation of polymorphonuclear leucocytes (PMN) by ligands f-MLP, PMA, or type I collagen. The inhibitory activity was localized in a peptide sequence comprising residues l85-203 of the non-collagenous domain (NC1) of the α3 chain of Type IV collagen (COL IV) (CNYYSWSYSFWLASLNPER)). The active site includes a triplet –Serine-Asparagine-Serine– (residues 189-191) (1). The receptors binding the peptide α3(IV) 185-203 as well as a longer sequence (residues 179-208) have been identified as CD47/αvβ3 integrin complex (2). It is suggested that during transmigration of PMN from the vascular lumen to the interstitial space, basement membranes are protected from damage by PMN through the α3 chain of COL IV (3).

In later studies we have shown that the same synthetic peptide, α3(IV)185-203, inhibits the replication of tumor cells by 67% and promotes their adhesion (70% over control) on a substrate of the same peptide (4). These studies again confirmed the requirement of the –SNS- triplet in the biological activity of the α3(IV) peptide. Replacement of serine in position 189 or 191 by alanine resulted in significant reduction in the ability to inhibit replication or promote adhesion of tumor cells. Treatment of the α3(IV)185-203 peptide with a mAb against the sequence α3(IV)179-203, with specific recognition of the triplet –SNS-, decreased almost completely (by 97%) the ability of the peptide to inhibit cell proliferation. Parallel studies showed that exposure of the tumor cells to intact COL IV or the bioactive peptide increased intracellular cAMP which was sensitive to pertussis toxin. cAMP analogues mimicked the effect of the α3(IV) peptide and Rp-cAMPS, a cAMP inhibitor, suppressed the inhibitory effect of COL IV and of the cAMP analogues. A protein kinase A inhibitor, H-89, blocked the inhibitory effect of COL IV and the α3(IV) peptide (5) The peptide also inhibits expression of metalloproteinases in tumor cells (6). Treatment of tumor cells with monoclonal antibodies against the receptors CD47 (Integrin Associated Protein) and the αvβ3 integrin inhibited their adhesion to the α3(IV) peptide, and prevented the ability of COL IV and of the α3(IV) peptide to inhibit tumor cell replication (2). The same antibodies prevented increased mobilization of intracellular $Ca^{2+}$ by the α3(IV) peptide (7).

Recent studies using the endothelial cell tube formation and the CAM models showed that the active peptide inhibits angiogenesis *in vitro*.

These studies suggest that COL IV through its α3(IV) chain inhibits tumor cell proliferation by binding to the receptors CD47/IAP and the αvβ3 integrin and activating the signal transduction pathway.

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