Titolo del programma

Ruolo delle isoforme 1 e 2 della proteinchinasi Akt nella invasivita' e motilita' di cellule di tumore prostatico umano.

Relazione conclusiva dell'attività di ricerca svolta dal Prof. Alex Toker, presso l'Istituto IGM-CNR di Bologna, nell'ambito del programma "short term mobility 2009" del CNR, periodo 1-10 dicembre 2009.

Bologna 10 02 2010

Premessa

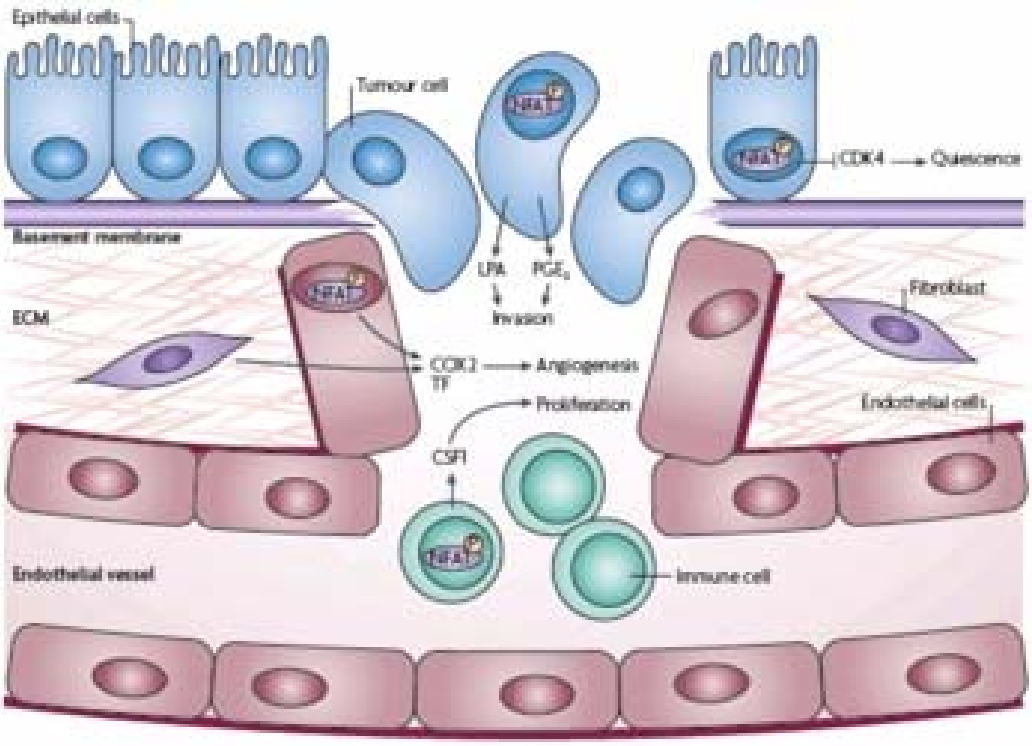
L'obiettivo che il programma si e' proposto e' determinare il ruolo specifico delle diverse isoforme di Akt nel promuovere la motilita'/invasivita' di cellule Pnt1a di tumore prostatico umano, utilizzando vettori lentivirali che codificano shRNA specifici per Akt 1 o Akt2 in saggi di migrazione/invasione cellulare in vivo e analizzare i substrati di Akt coinvolti tramite proteomica funzionale. Inoltre, un secondo obiettivo e' stato investigare il ruolo della proteina clusterina nella modulazione dell'espressione di Akt 1 e 2 e quindi della motilita' di cellule Pnt1.

Come previsto, l'attivita' svolta dal dr. Toker ha riguardato l'allestimento di saggi di migrazione/invasione di cellule pnt1A wild type o overesprimenti clusterina, dopo down-modulazione delle singole isoforme di Akt con specifci shRNA. Inoltre, e' stato valutato lo stato di fosorilazione dei componenti della via di segnale di Akt al fine di individuare gli intermedi di segnale responsabili della risposta ad Akt.
Il razionale del progetto era basato sulla recente scoperta fatta nel laboratorio del dr. Toker sul ruolo del proto-oncogene proteinchinasi B/Akt nella progressione del tumore della mammella, che si puo' riassumere nella conoscenza che l'isoforma Akt1 funziona da soppressore della motilita' delle cellule cancerose, ostacolando l'invasione e la formazione di metastasi, mentre l'isoforma Akt2 promuove la motilita' cellulare e l'invasivita'. Questa scoperta e' tanto piu' rilevante quanto inattesa, considerando che la via di segnale governata da PI3-chinasi/Akt e' chiaramente implicata nella progressione tumorale, e numerose compagnie farmaceutiche stanno sviluppando piccole molecole inibitrici di Akt da utilizzare nelle terapie antitumorali, alcune delle quali gia' in trial clinico.

Background

1. Akt and cancer cell migration.

A major research focus in the laboratory of dr. Toker is the regulation of carcinoma cell invasive migration, with emphasis on the signaling pathways which impact this phenotype (fig. 1). We are investigating the mechanisms by which the PI 3-kinase and Akt signaling pathway regulates breast cancer progression. Genes in the the PI 3-K pathway harbor some of the most frequent genetic lesions in breast cancer. Our studies have focused on the role of the Akt kinase in modulating breast cancer progression. We have discovered that Akt1 is breast cancer cell motility and invasion suppressor, a surprising finding considering that the PI 3-K and Akt pathway is clearly implicated in tumor progression. Conversely, the Akt2 isoforms is an enhancer of invasive migration and metastatic dissemination. The significance of these findings is that small molecule inhibitors of Akt, currently being developed by many pharmaceutical companies, may actually enhance tumor invasion and metastasis. We are currently investigating the mechanisms by which Akt isoforms control invasive migration in vitro and in vivo, as well as conducting phospho-proteomic screens for novel Akt substrates, and evaluating the role of the related SGK kinase in breast cancer progression.



(figure 1)

2. *Clusterin and prostate cancer*. Clusterin (CLU), is a secretory heterodimeric disulphide-linked glycoprotein (449 amino acids) that is expressed in virtually all tissues, and found in all human fluids.

CLU is an enigmatic molecule, implicated in diverse biological processes, and has additionally been associated with opposing functions in regard to apoptosis. Accumulating evidence from several studies suggests that the pro- and anti-apoptotic

functions may be related to nuclear and secreted protein isoforms, respectively. The secreted form of CLU is a glycosylated protein of 70–80 kDa that consists of two chains held together by five disulfide bonds, and consequently it appears as a ~40 kDa smear on immunoblots from reducing SDS-PAGE. Its intracellular pre-curser form of 60 kDa may also exhibit an antiapoptotic function. The proapoptotic CLU variant is a 50–55 kDa protein which accumulates in the nucleus of apoptotic cells. How these different CLU protein variants are produced from the CLU gene is poorly understood, although it has been speculated that nuclear CLU results from an alternative splice event skipping exon 2 from the main CLU transcript otherwise translated into secreted CLU.

Recently, it has been reported that CLU reduces the migratory properties of vascular smooth muscle cells as well as of endothelial cells. This suggests that CLU might also be involved in the control of the metastatic properties of tumor cells. The proposed research is aimed at verifying whether clusterin and Akt play some role in the control of the metastatic properties of tumor cells.

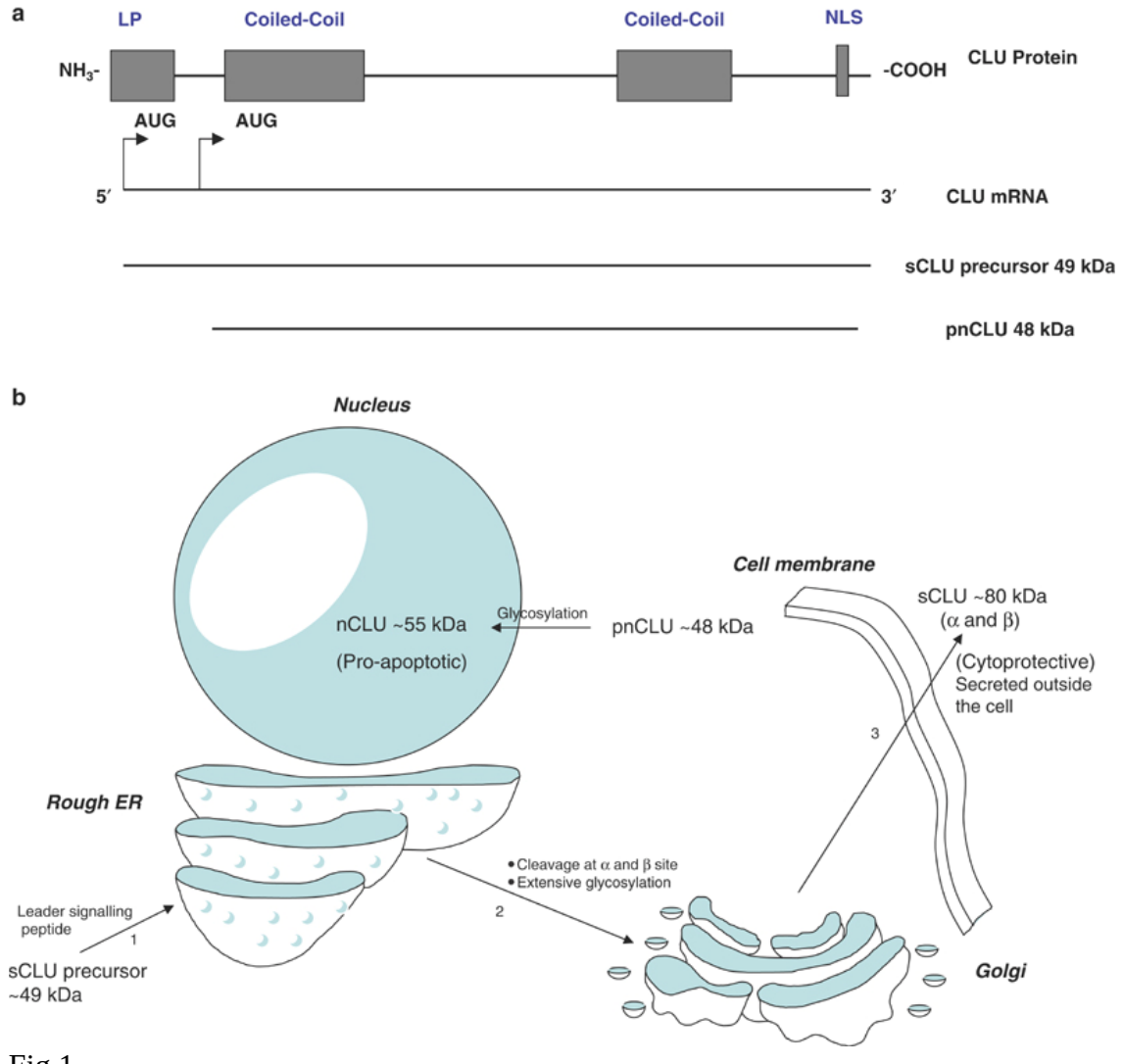


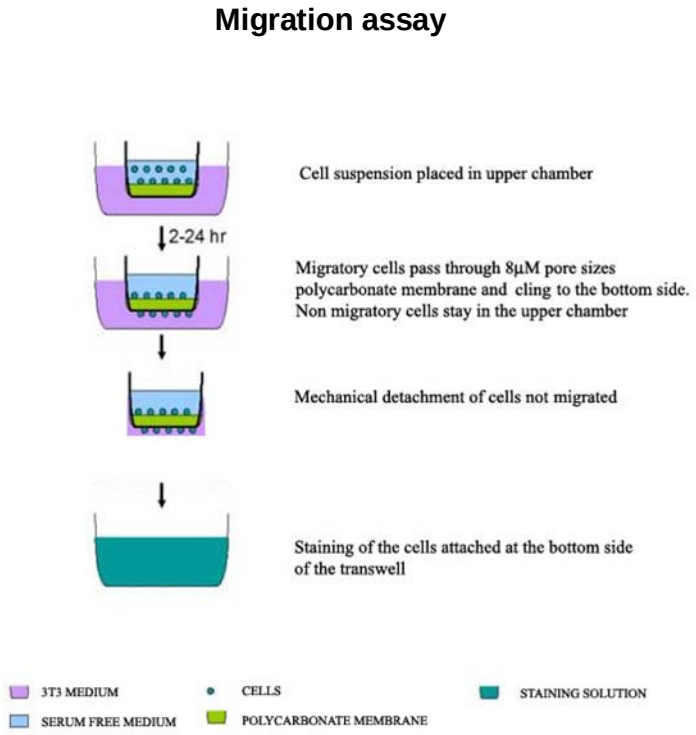
Fig.1

On these basis, the work done during the 2 weeks at IGM-CNR in Bologna focused on the possible role of Akt 1/2 in modulating migration/invasion of human prostate cancer PNT1A overexpressing clusterin.

Results and conclusions

The migration assay was performed as illustrated in figure 1:

Fig.1



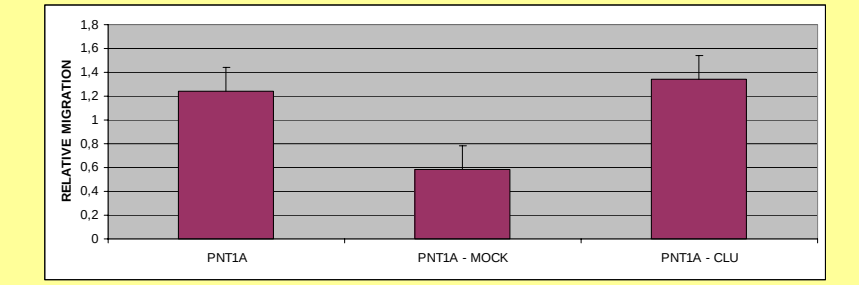
As shown in figure 2, the migration ability of PNT1A wild type cells is dramatically increased by the overexpression of full length clusterin (PNT1A clu cells). Since our previous data indicate that PNT1A clu cells display different amounts of Akt1 and 2 with respect to wild type PNT1A cells, and due to the recently revealed opposite functions of the two Akt isoforms on cell migration, we aimed to determine whether the much higher expression and phosphorylation of Akt1 in PNT1A clu cells is responsible for their migratory phenotype.

Therefore, wild type PNT1A cells and PNT1A clu cells (stably transfected) were infected or not with pLko lentiviral vector expressing shRNA either Akt1-or Akt2-specific, for 72 h in the presence of puromycin, to down-modulate Akt1 or Akt2 expression, respectively. Western blots were run to monitor infection efficiency.

However, as shown in figure 3 and 4, while down-regulation of Akt1 increases the migration of both wild type PNT1A cells and PNT1A clu cells, down-regulation of Akt2 by specific shRNAs decreases the migration of both wild type PNT1A cells and PNT1A

clu cells, ruling out that the differential expression and activity of the two Akt isoforms can influence the migratory potential of PNT1A cells.

Fig. 2



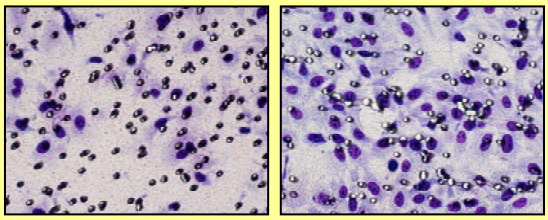


Fig. 3

The downregulation of Akt1 increases the migration ability of both clones

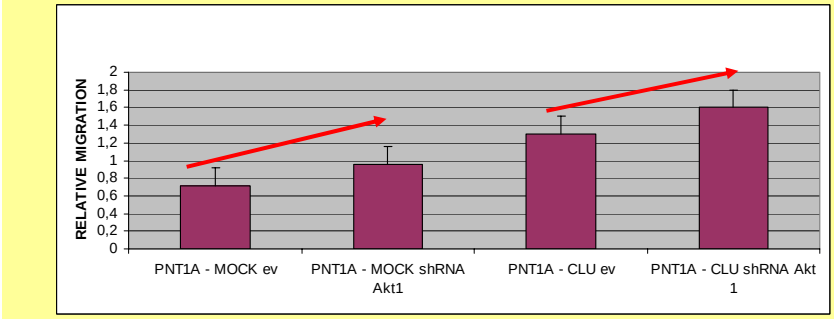


Fig. 4

The downregulation of Akt2 decreases the migration ability of both clones