Mesenchymal, Endothelial and Immune Cells as Pathogenetic and Therapeutic Targets of Lung Cancer

D. Madeddu[1], A. Falco[1], B. Lorusso[1], C. Frati[1], G. Graiani[1], F. Saccani[1], A. Gervasi[2], P. Rossetti[1], F. Ricci[1], G. Bocchialini[1], L. Ampollini[3], L. Gnetti[2], CA. Lagrasta[2], E.M. Silini[2], E. Quaini[4], P.G. Petronini [1], R. Alfieri[1], P. Carbognani[3], F. Rossi[5], A. De Angelis[5], K. Urbanek[5], F. Quaini[1]

Depts. of [1]Clinical and Experimental Medicine, [2]SBIBIT and [3]Surgical Science, University-Hospital of Parma, Parma, Italy
[4]Institute of Life Science , Scuola Superiore Sant'Anna, Pisa, Italy
[5]Experimental Medicine, Pharmacology, Second University of Naples, Naples - Italy

Advanced therapies have not been able to determine robust positive results on survival in patients with lung cancer (LC). The existence of Cancer Initiating Cells (CICs), able to develop tumors upon serial xenotransplantation, opens new avenues in the pathogenetic mechanism, therapy resistance and disease progression in LC. Importantly, CIC may reside in protective niches that allow a proper balance between self-renewal and differentiation. Endothelial cells (EC) and Mesenchymal Stromal Cells (MSC) constitute an essential component regulating CICs within the niche through a tight control of local O_2 and the release of growth factors. Moreover, blood and lymphatic vessels closely interact with CICs, constituting the vascular niche. Although genetic mutations represent a relevant tumorigenic event, whether this phenomenon provides self-autonomy to cancer cells to develop tumors is questionable. Indeed, the limited success of molecularly targeted drugs points to the tumor microenvironment as an additional oncogenic drive in LC. Finally, the recent therapeutic success of antibodies targeting PD-1/PDL-1 immune checkpoints in LC highlights the role of the immune system.

The aim of the present work was to characterize at cellular and tissue level the actors and competitors of the initiating and promoting events implicated in LC development.

To this purpose, 20 fresh samples from LC and spared distant lung (DL) were processed for MSC and EC isolation, expansion and characterization. Their involvement in LC growth was tested in vitro by co-culture and conditioned media, and in vivo in a mouse model of xenotransplantation. Sections from the same LC patients were immunostained for the detection of cells expressing: 1) stem cell related antigens (CD44, CD133, CD117); 2) transcription factors involved in stemness (OCT3/4, SOX2) and bronchio-alveolar lineage commitment (TTF-1, Ets-1); 3) PDGFr and CD34. The incidence of lymphatic (LYVE-1, Podoplanin) and hematic (CD31, vWF, α-SMA) vascular structures was also measured. Moreover, morphometric quantification of tumor cells expressing PDL-1 and PD-1 labelled tumor infiltrating lymphocytes (TILs) was assessed.

Results indicated that in vitro growth and mitotic index of LC-MSC and LC-EC were reduced compared to corresponding DL derived cells. LC-MSC display higher expression of OCT3/4, SOX2 and TTF-1 and more efficiently promoted both in transwell assay and by contact human LC cells (Calu-3 line) proliferation than DL-MSC. Comparative PCR array analysis showed that specific inflammasome (CXCR4, CCL12, IL1A, TLR2-4) and apoptotic (BCL2) pathways were differentially regulated in the two MSC populations. Xenotransplantation in Balb/c Nude mice of Calu-3 alone or with MSCs documented that LC-MSC significantly increased Calu-3 replication in vivo and thereby tumor volume while DL-MSC had marginal effects. Cell tracking documented that MSC were bordering neoplastic nodules within the tumor xenografts. In vitro investigations on ECs showed that LC derived hematic and lymphatic cells display a different morphology and pattern of expression of VEGFR, PDGFR and c-met, and ability to form tubule-like structures on Matrigel compared to DL-ECs. The immunohistochemical and morphometric analysis of neoplastic tissues from primary LCs, demonstrated a trend towards a positive gradient from remote to proximal areas of cells carrying CD117 or vascular progenitors (PDGFR, CD34) markers. Importantly, a high heterogeneity both in PDL-1 expression by tumor cells and in amount of $PD1^{pos}$ TILs was present among patients and within different areas of the same tumor. The potential correlation of these important immune regulatory parameters, and biological properties of MSC and EC with the clinical characteristic of LC patients is under intense investigation.

Studies aimed at the development of specific targeted therapeutic strategies may be designed to interfere with the tumor microenvironment and with the L-CIC niche.