La gioia di vivere,

P. Picasso, 1946

Olio su cemento-amianto

Nuovi marcatori bioumorali nella diagnosi precoce di patologie asbesto correlate

Prof.ssa M. Comar
UCO Igiene e Medicina Preventiva
Università degli Studi di Trieste

'A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to a therapeutic intervention'

(National Institutes of Health's Biomarkers Definitions Working Group)

- La diagnosi precoce del Mesotelioma Maligno è estremamente difficile in quanto solo il 5% dei pazienti è diagnosticato nello stadio 1°A della malattia .

- È critica la necessità di individuare un sistema non invasivo ed efficace in grado di ridurre il ritardo diagnostico.

- Ad oggi non sono disponibili un marcatore o pannello di marcatori solubili standardizzati per una diagnosi certa di Mesotelioma.

Marcatori con ruolo diagnostico

- Hyaluronic acid
- Ca12
- Ca-15.3
- TPA
- Cyfra 21.1
- **OSTEOPONTIN (OPN) (low specificity)**
- **MEGAKARYOCYTES POTENTING FACTOR (MPF) (alternative to SM)**
- **FIBULIN-3 (2014-prognostic marker)**
- **MESOTHELIN (SM) (FDA-approved biomarker)**

Rilevanti solo nel contesto di associazione con l'outcome della malattia

Un marcatore ideale per il mesotelioma

- Diagnosi:

-individuare i diversi istiotipi tumorali

-differenziare il MPM da iperplasia mesoteliale e adenocarcinoma metastatico

- Definire il grado di progressione

- Monitoraggio del trattamento/ remissione

- Fattore prognostico in assenza di trattamento

- **Predizione della malattia nei soggetti esposti all'asbesto.**

PROTEINE INFIAMMATORIE NEL MM

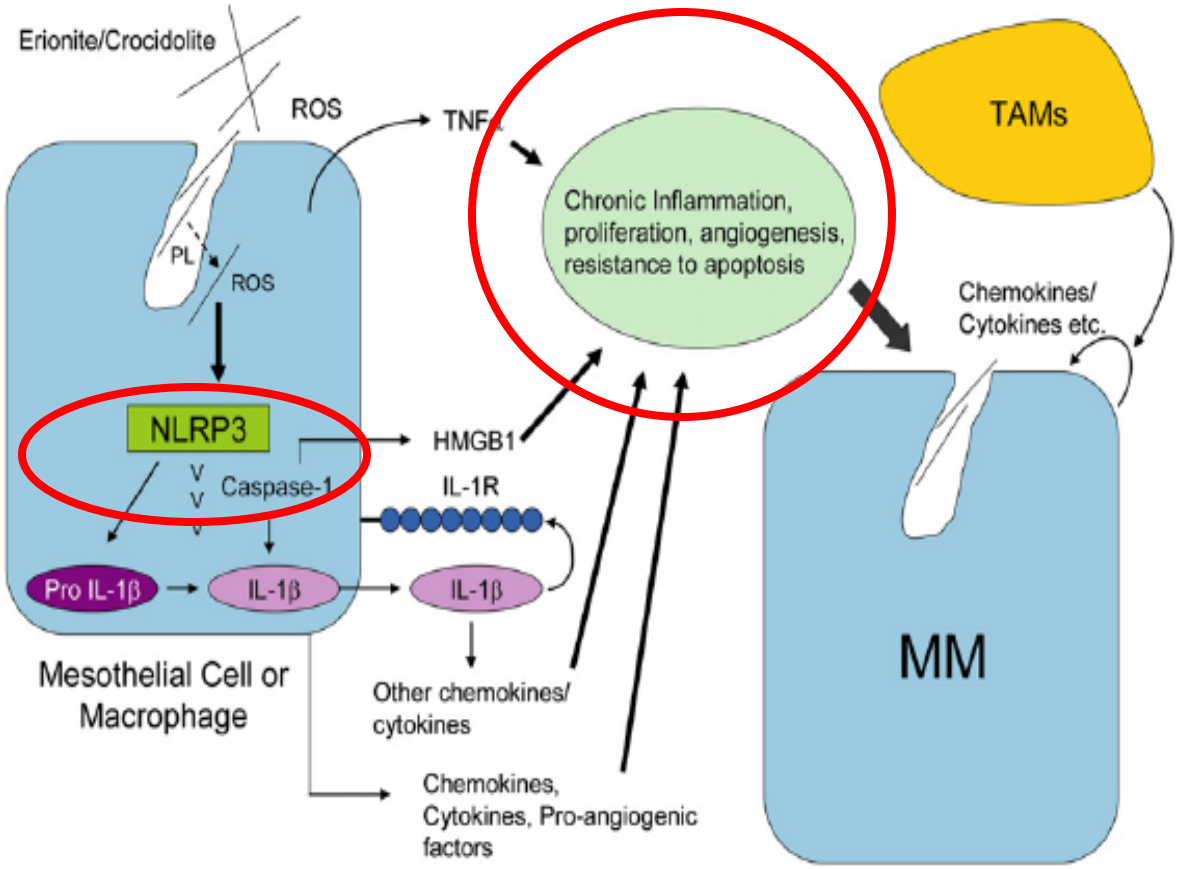


Figure 4 The NLRP3 (NAPL3) inflammasome is a key player in initiation of inflammation and release of chemokines and cytokines in human mesothelial cells and macrophages in response to long, pathogenic fibers. ROS appear to play a role in both activation of NADPH during phagocytosis and lysosomal degradation, which then releases asbestos fibers into the cytoplasm, where they interact with NLRP3 and induce caspase-1 activity. As a consequence, mature IL-1β, high-mobility group protein 1, and IL-1β–related cytokines are released into the tumor milieu, creating episodic bouts of cell injury, inflammation, and compensatory proliferation. Levels of these key inflammatory factors are reduced in mesothelial cells transfected with small-interfering NLRP3 and enhanced in the presence of TNF-α released by mesothelial cells, TAMs, and macrophages in the tumor environment.[49,67]

Girardelli et al. Infectious Agents and Cancer 2012, 7:25
http://www.infectagentscancer.com/content/7/1/25

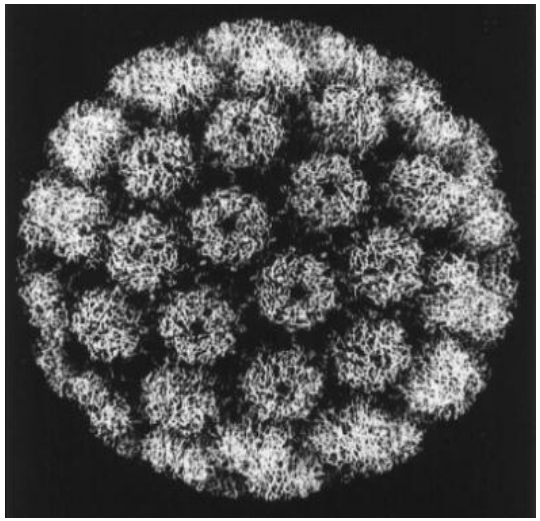
INFECTIOUS AGENTS AND CANCER

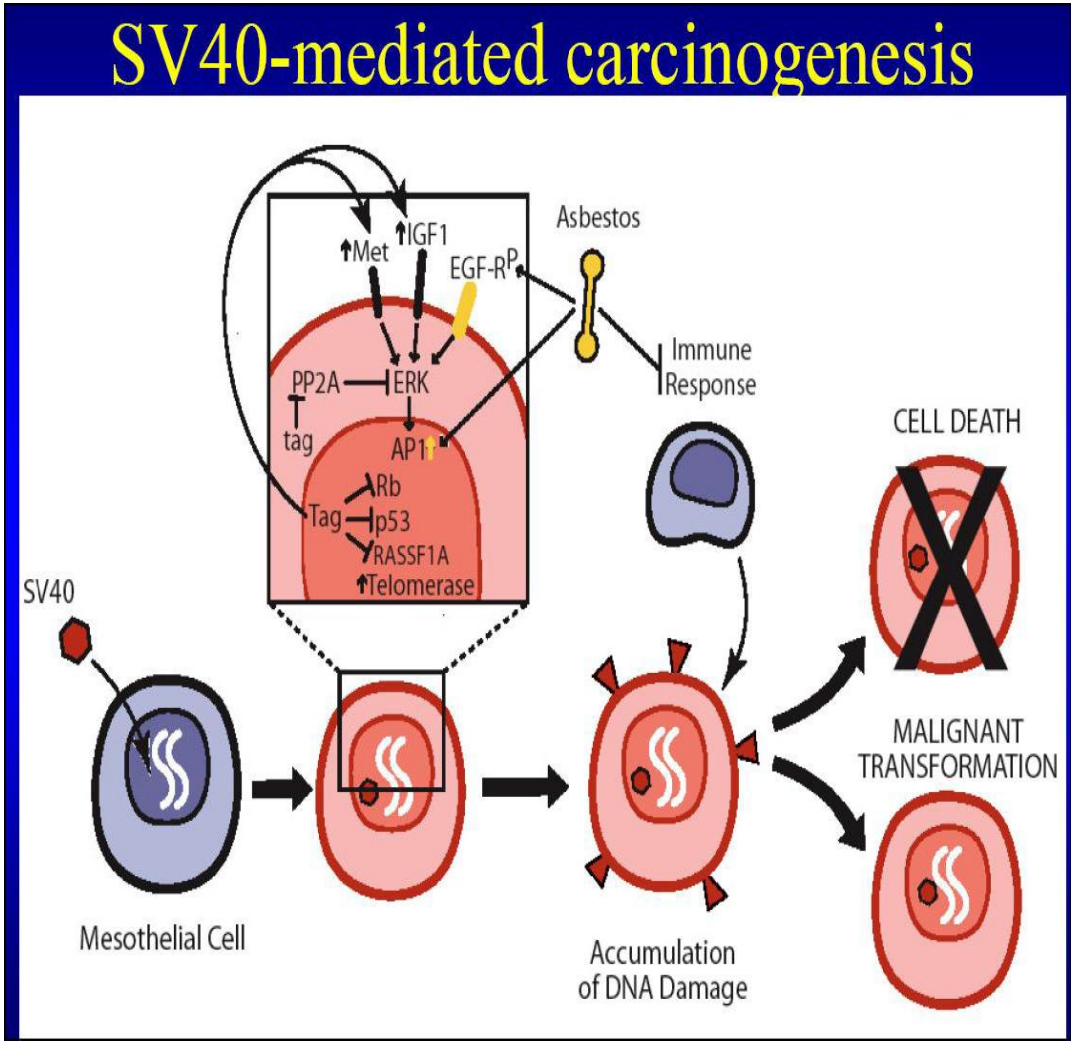
RESEARCH ARTICLE Open Access

NLRP1 polymorphisms in patients with asbestos-associated mesothelioma

Martina Girardelli[1], Iva Maestri[3], Rosa R Rinaldi[4], Mauro Tognon[5], Renzo Boldorini[6], Massimo Bovenzi[7], Sergio Crovella[2] and Manola Comar[2*]

SV40





SV40 aumenta il rischio di mesotelioma fra individui esposti ad asbesto in quanto incrementa gli effetti carcinogenetici di quest'ultimo: la penetrazione delle fibre di asbesto nelle cellule mesoteliali infette da SV40, non genera apoptosi grazie all'azione del virus

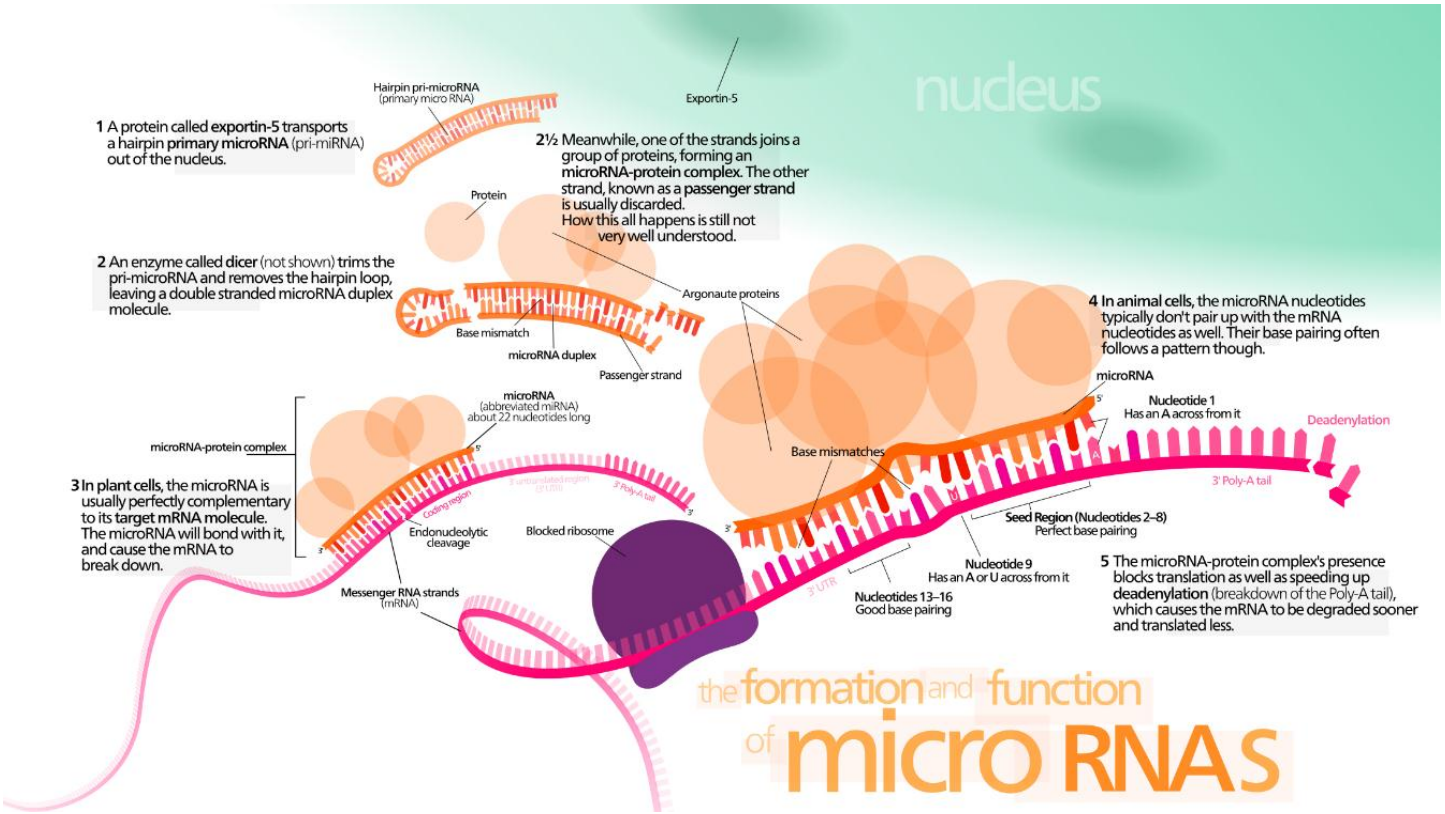
High prevalence of serum antibodies reacting with simian virus 40 capsid protein mimotopes in patients affected by malignant pleural mesothelioma

Elisa Mazzoni[a], Alfredo Corallini[b], Alfonso Cristaudo[c], Angelo Taronna[b], Gianfranco Tassi[d], Marco Manfrini[a], Manola Comar[e], Massimo Bovenzi[f], Roberto Guaschino[g], Francesca Vaniglia[h], Corrado Magnani[i], Ferruccio Casali[j], Giovanni Rezza[k], Giuseppe Barbanti-Brodano[b], Fernanda Martini[a,1,2], and Mauro G. Tognon[a,1,2]

Sections of [a]Cell Biology and Molecular Genetics and [b]Microbiology, University of Ferrara, 44121 Ferrara, Italy; [c]Section of Occupational Medicine, School of Medicine and Surgery, University of Pisa, 56124 Pisa, Italy; [d]Respiratory Diseases Clinic, United Hospitals, 25122 Brescia, Italy; [e]Institute for Maternal and Child Health–IRCCS "Burlo Garofolo"–Trieste and [f]Clinical Unit of Occupational Medicine, Department of Medical Sciences, University of Trieste, 34137 Trieste, Italy; [g]Blood Bank, City Hospital of Alessandria, 15121 Alessandria, Italy; [h]Blood Bank, City Hospital of Casale Monferrato, 15033 Casale Monferrato, Italy; [i]Section of Medical Statistics, School of Medicine and Surgery, University of East Piedmont, 28100 Novara, Italy; [j]Clinical Laboratory Analysis, City Hospital of the Republic of San Marino, 47892 Republic of San Marino; and [k]Istituto Superiore di Sanità, 00161 Rome, Italy

Edited* by George Klein, Karolinska Institute, Stockholm, Sweden, and approved September 19, 2012 (received for review July 31, 2012)

Micro RNA:



1. I **microRNA** (**miRNA)**, sono piccole molecole endogene di RNA non codificante, a singolo filamento.
2. 20-22 nucleotidi
3. I miRNA funzionano tramite accoppiamento-base con sequenze complementari delle molecole di mRNA
4. Il genoma umano codifica per più di 2000 miRNA.

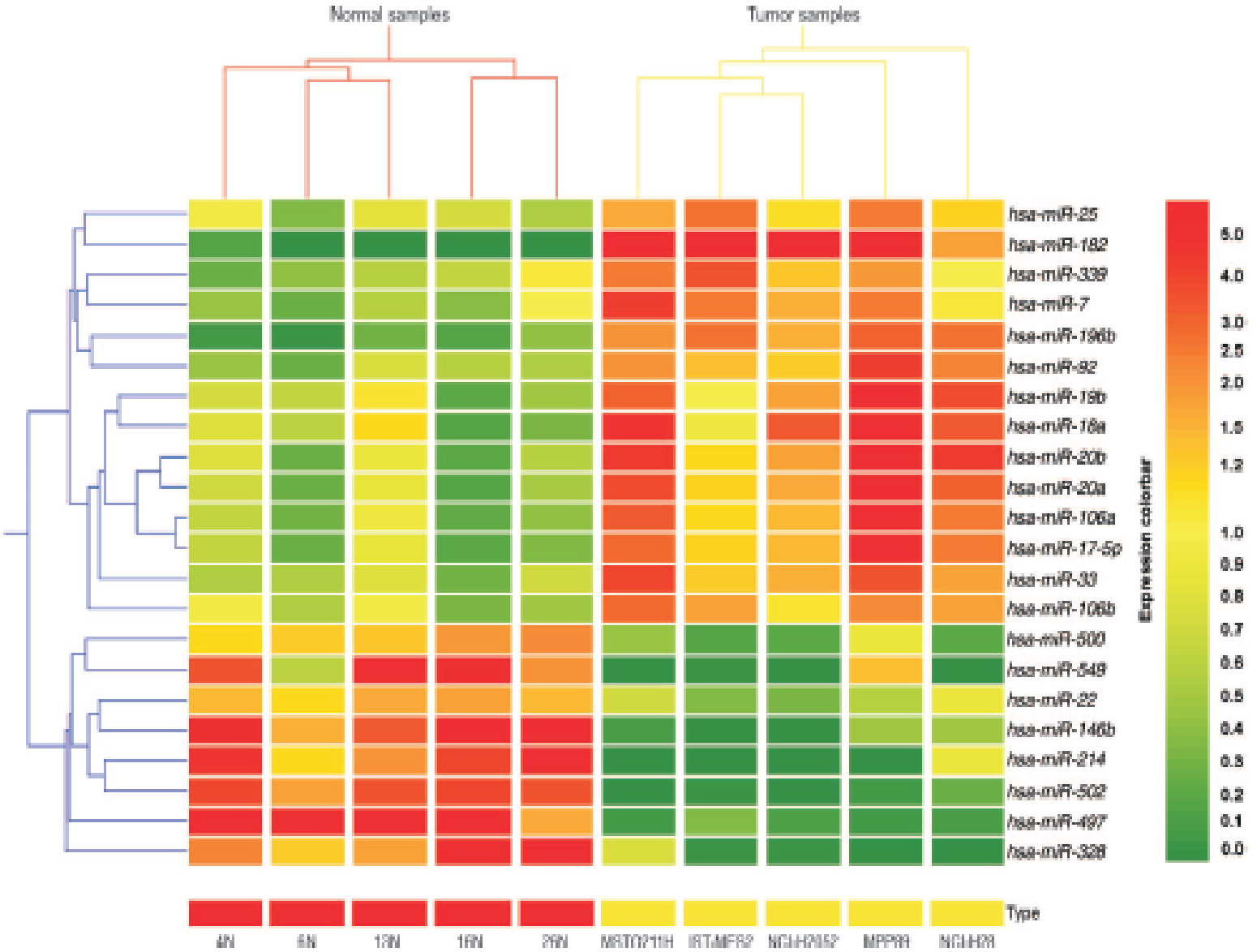


FIGURE 1. Cluster analysis of microRNA (miRNA) expression profiles in human normal pleural mesothelial short-term cell culture (HMC) and malignant pleura mesothelioma (MPM) cells. Cluster analysis obtained using the 22 miRNAs described in Table 1, which exhibit at least a twofold differential expression between MPM and HCM samples, and which a p value <0.05 was applied to. The red color represents normal samples, whereas yellow represents tumors.

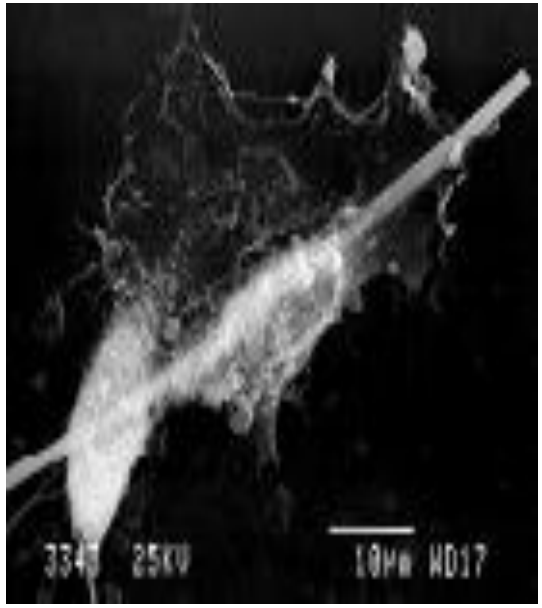
Patogenesi: inalazione delle fibre

Il 70% delle fibre inalate viene eliminata (espettorato).
Il 30% attraversa l'epitelio alveolare penetrando nell' interstizio polmonare.

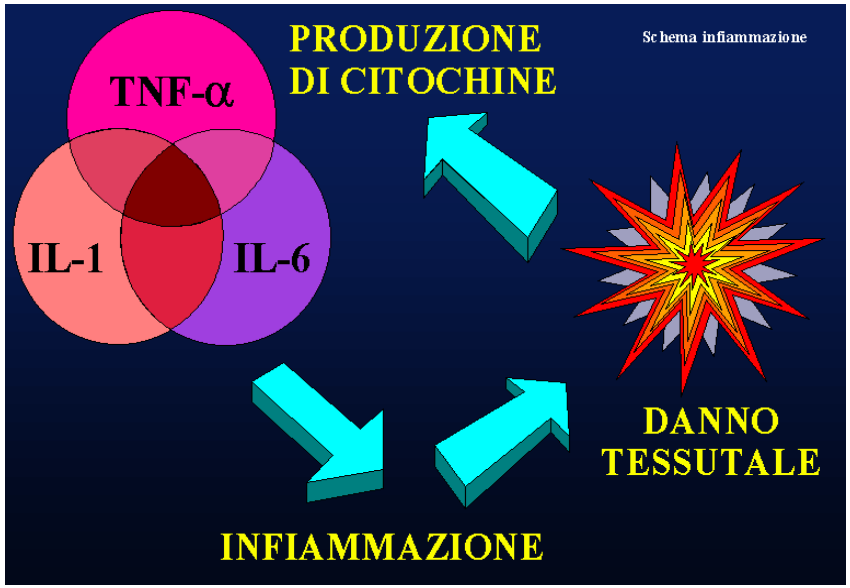
attivazione dei macrofagi alveolari

produzione di citochine

- deregolazioni di protooncogeni (PDGFb)
- conseguente crescita mesoteliale



Citochine come biomarcatori solubili per la patologia asbesto indotta ?



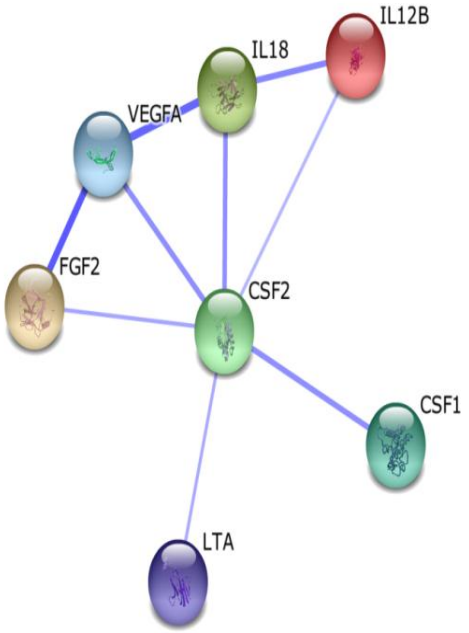


Table 2. Serum concentration of significative cytokines in MM patients, Abs-workers and healthy subjects.

Cytokines	MM Patients (n°15)	Abs-workers (n°15)	Healthy subjects (n°13)
IL-1α***	67.37 (0.35–75.03)[f,h]	0.35 (0.35–0.35)	0.01 (0.01–0.01)
IL-2Ra**	51.62 (45.79–68.29)[e]	18.69 (0.13–33.83)[b]	152.30 (126.10–192.24)
IL-3**	63.68 (0.46–66.63)[f,h]	0.46 (0.46–0.46)	0.46 (0.46–0.46)
IL-12p40**	216.53 (1.17–229.62)[f,h]	1.17 (1.17–1.17)	1.17 (1.17–1.17)
Ctack*	829.24 (133.79–1028.74)[h]	108.30 (89.08–120.14)	250.39 (172.92–384.29)
GROα**	13.95 (0.47–19.68)	0.47 (0.47–0.47)[c]	24.17 (15.39–30.41)
HGF**	37.87 (17.05–59.59)[f]	30.54 (30.43–30.64)[b]	228.98 (164.78–494.60)
IFN-α2*	0.09 (0.09–14.93)	9.98 (7.85–17.07)[a]	0.09 (0.09–0.09)
MCP-3***	13.91 (0.18–15.01)[f,h]	0.18 (0.18–0.18)	0.18 (0.18–0.18)
M-CSF***	1.04 (0.44–2.18)[f]	0.44 (0.44–0.44)[b]	10.39 (9.34–12.50)
MIF*	2.33 (1.06–28.25)[d]	1.06 (1.06–1.06)[a]	33.40 (19.13–41.67)
MIG**	70.87 (23.73–518.81)[d]	432.49 (396.93–466.37)	1032.23 (162.00–1876.80)
β-NGF***	36.51 (0.26–36.56)[f,h]	0.26 (0.26–0.26)	0.26 (0.26–0.26)
SCGF-β***	1386.14 (3.16–1782.43)[e]	3.16 (3.16–3.16)[c]	6059.88(4970.20–50221.58)
SDF-1α***	3.33 (0.57–7.18)[f]	0.57 (0.57–0.57)[c]	20.92 (19.90–22.40)
TNF-β***	20.00 (0.36–20.29)[f,g]	0.36 (0.36–0.36)	0.36 (0.36–0.36)
IL-4***	3.40 (2.49–5.57)[f]	1.90 (1.44–2.39)[c]	7.09 (6.70–7.59)
IL-6*	16.91 (11.32–34.11)[g]	6.06 (0.38–11.63)	10.63 (7.94–12.21)
IL-8***	17.13 (13.63–24.24)[e,g]	10.77 (0.40–12.48)[c]	27.10 (22.79–30.51)
IL-17A*	59.01 (28.85–93.80)	28.99 (0.44–29.87)[a]	96.08 (88.64–115.72)
EOTAXIN*	0.48 (0.48–87.25)[g]	139.62 (95.31–223.62)[b]	24.30 (0.48–31.20)
G-CSF*	19.80 (13.51–42.33)	12.91 (10.25–13.36)	39.80 (28.70–54.57)
IFN-γ**	169.01 (54.64–230.36)[h]	38.71 (19.24–45.01)[a]	135.47 (127.89–149.47)
IP-10	1432.13 (522.52–1909.76)	851.81 (631.41–279.92)	484.73 (380.98–842.63)
MIP-1α***	2.76 (0.30–3.44)[f]	0.30 (0.30–0.30)[a]	7.10 (6.12–7.46)

La C-C chemochina RANTES è associata all'esposizione all' asbesto

OPEN ACCESS Freely available online

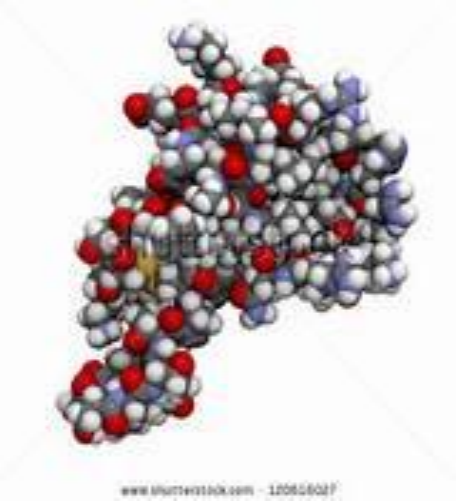
PLOS | ONE

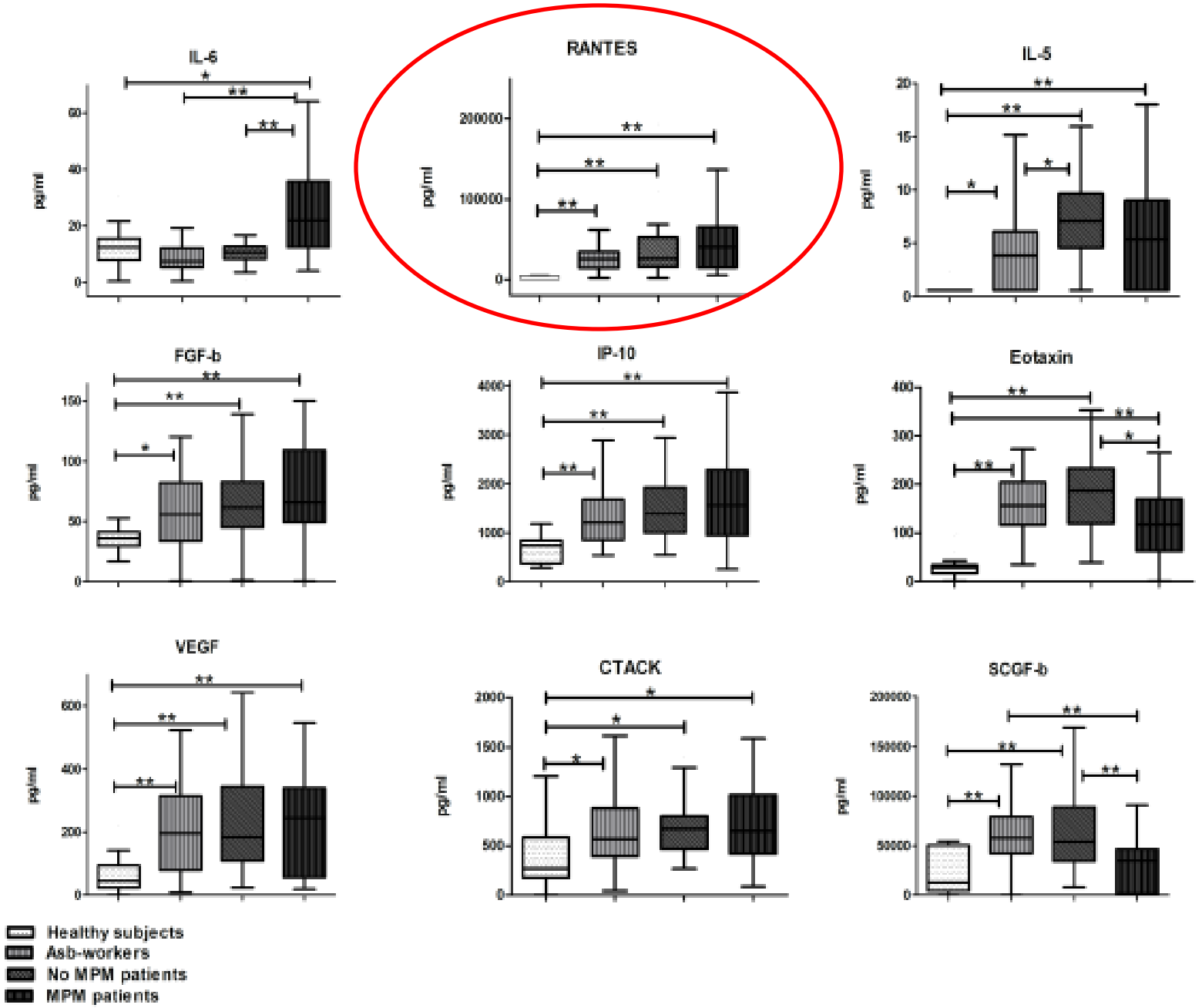
Increased Levels of C-C Chemokine RANTES in Asbestos Exposed Workers and in Malignant Mesothelioma Patients from an Hyperendemic Area

Manola Comar[1,2]*, Nunzia Zanotta[1], Alessandra Bonotti[3], Mauro Tognon[4], Corrado Negro[5], Alfonso Cristaudo[3], Massimo Bovenzi[5]

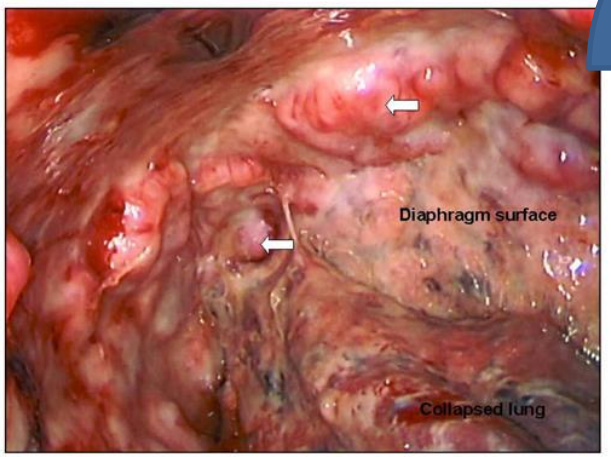
1 Institute for Maternal and Child Health-IRCCS "Burlo Garofolo"–Trieste, Trieste, Italy, **2** Clinical Unit of Hygiene, Department of Medical Sciences, University of Trieste, Trieste, Italy, **3** Division of Occupational & Preventive Medicine, University Hospital of Pisa, Pisa, Italy, **4** Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, School of Medicine, University of Ferrara, Ferrara, Italy, **5** Clinical Unit of Occupational Medicine, Department of Medical Sciences, University of Trieste, Trieste, Italy

Un aumentato livello sierico di RANTES è indice di attività pro-tumorale associata all'esposizione contribuendo alla progressione della malattia ??

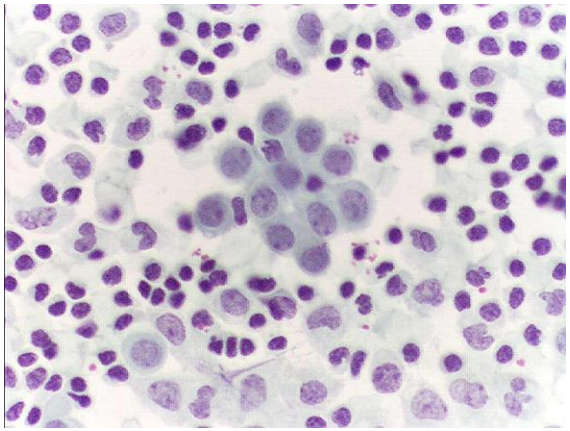



IL-6
RANTES
IL-5
FGF-b
IP-10
Eotaxin
VEGF
CTACK
SCGF-b
pg/ml
Healthy subjects
Asb-workers
No MPM patients
MPM patients

RANTES



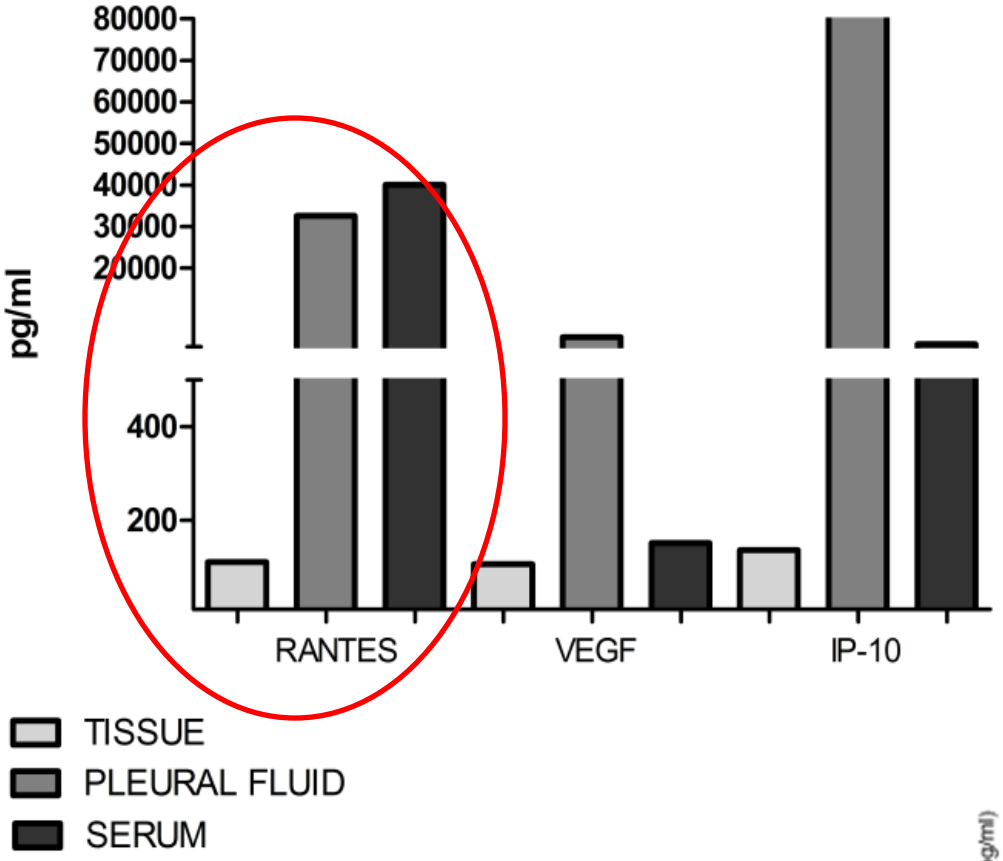
Mesotelioma



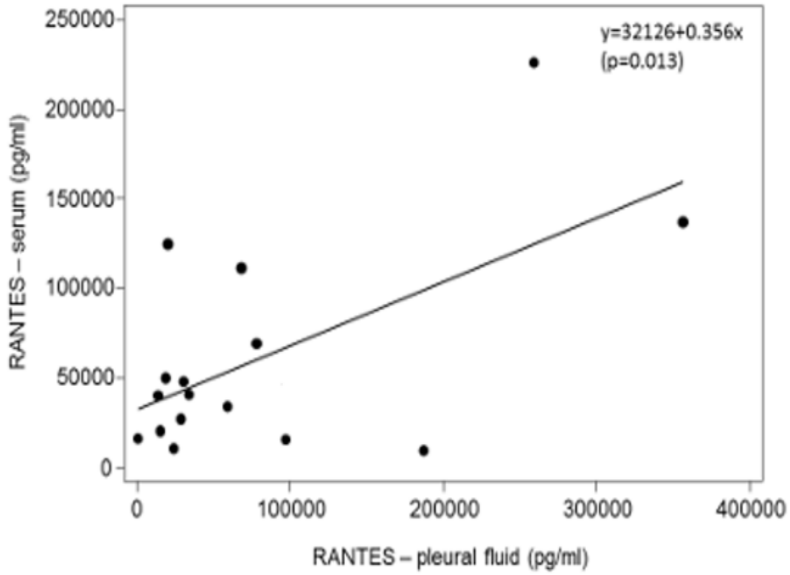
Frazione cellulare
Liquido pleurico

Siero

Correlazione tra siero, liquido pleurico e tessuto tumorale provenienti dallo stesso paziente

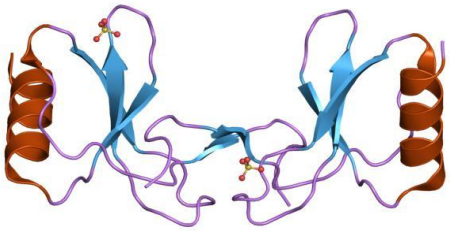

pg/ml
80000
70000
60000
50000
40000
30000
20000
400
200
RANTES
VEGF
IP-10
TISSUE
PLEURAL FLUID
SERUM

RANTES


y=32126+0.356x
(p=0.013)
250000
200000
150000
100000
50000
0
RANTES – serum (pg/ml)
0
100000
200000
300000
400000
RANTES – pleural fluid (pg/ml)

Cytokines in patients with benign asbestos induced-diseases and malignant pleural mesothelioma: association of RANTES growth factor with asbestos exposure and diseases severity.

Comar et al 2015 (submitted)



Chemokine (C-C motif) ligand 5

Prof. M. Bovenzi
Medicina del Lavoro
Azienda Ospedaliera-Universitaria di Trieste.

Dott. M. Cortale
Chirurgia Toracica
Azienda Ospedaliera-Universitaria di Trieste

Prof. A. Cristaudo
Medicina del Lavoro
Università degli Studi di Pisa

Prof. M. Tognon
Biologia cellulare e genetica molecolare
Università degli Studi di Ferrara.

REGIONE AUTONOMA FRIULI VENEZIA GIULIA

Pazienti
Lavoratori Ex esposti
Famiglie