Arginase-2 and immune response in non-small-cell lung cancer

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INTRODUCTION

L-Arginine (Arg) is a conditionally essential aminoacid that plays an important role in energy metabolism and signaling pathways. Arginine deprivation in normal cells leads to cell cycle arrest and quiescence, while cancer cells may continue their growth and activate apoptosis. Arginases (ARG) convert Arg to Ornithine and urea. ARGs exist in two distinct forms, the Type I (ARG-1) that is cytosolic and is mainly expressed by hepatocytes, and the Type II (ARG-2) that is located in the mitochondrial matrix and is expressed in many tissues.

Arg is essential to T-lymphocytes as it supports the expression of T-cell receptors (TCR) by regulating the translation of the ζ-chain peptide and the subsequent activation of CD4 and CD8 T-cells**.** As arginine auxotrophy (exogenous-Arg consumption) widely characterizes tumors, it is postulated that Arg-depleted tumor microevironment is a critical condition blocking T-cell cytotoxic activity**.**

In this study we examined the expression of ARG-2 and its correlation with markers of intratumoral hypoxia and markers of immune response.

PATIENTS

No pts	98
Age (median/range)	
68(32-82)	
Histology	
Squamous	58
Adenocarcinoma	22
Undifferentiated	18
Stage	
I	46
II	22
III	30
Follow-up (median months)	46
Treatment	Surgery

ARG-2 IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded tissue sections of 3μm thick were placed on positively charged slides. For the detection of arginase-2 (ARG-2) we used the primary rabbit polyclonal ab191029 antibody (abcam, UK), with 60 min incubation at dilution 1/1000. The DAKO Envision Kit was used.

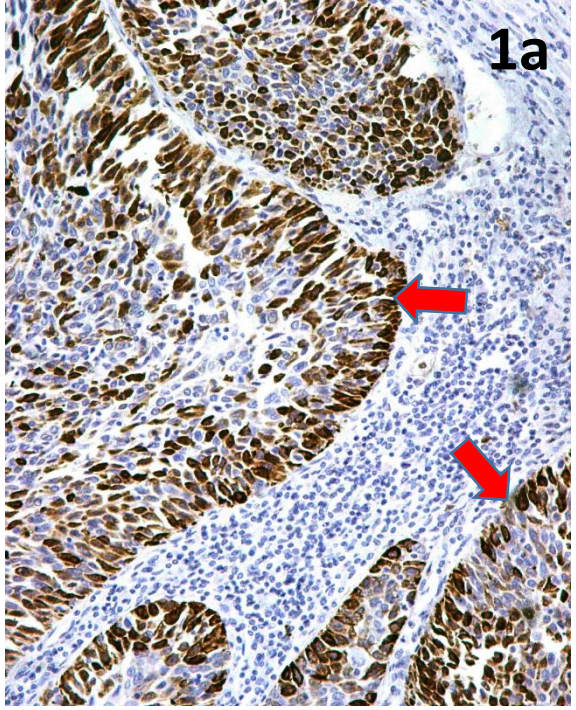
Assessment of the expression of ARG-2 was performed at x200 magnification. The percentage of cancer cells with strong cytoplasmic expression was recorded in all optical fields and the mean value was calculated and used to score each tissue section.

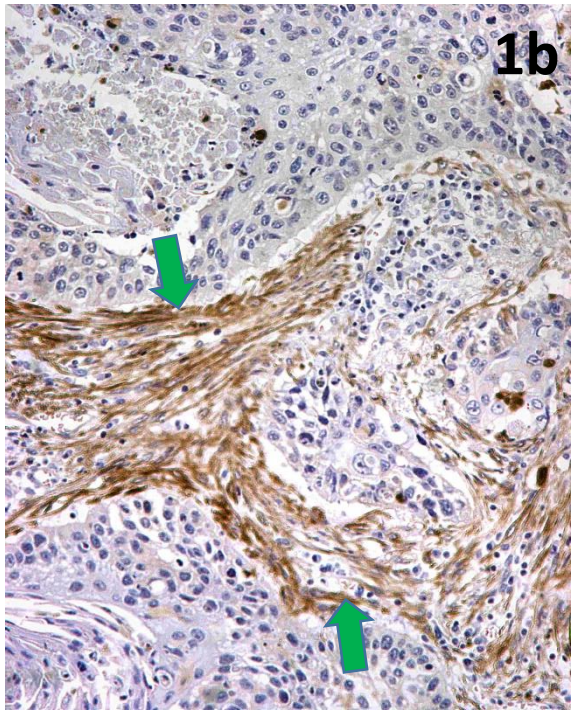
The extent of cancer associated fibroblasts (CAFs) stained with the ARG-2 Abs was recorded as the percentage of stained stroma area in the tissue section, at x200 magnification.

Tumor infiltrating lymphocytes (TILs) were quantified on hematoxylin/eosin stained tissue sections (low to high score:1 to 4).

Statistical analysis was performed using the GraphPad Prism 7.0.

REFERENCES: Tapiero H et al. Biomed Pharmacother 2002;5:439-45; Wheatley DN. Anticancer Drugs. 2004;15:825-33; Riess C etal Cell Physiol Biochem. 2018;51:854-70; Bronte V, et al Trends Immunol. 2003;24:302–6





RESULTS

Bronchial and alveolar epithelium, as well as glandular epithelium had a very weak ARG-2 expression.

ARG-2 was strongly expressed in the cytoplasm of 25/98 cases (10-90% of the cancer cell population, median 40). Expression in less than the median was recorded in 13/98 (13.3%) cases (medium expression group), whilst extensive expression in 50-90% of cells was recorded in 12/98 (12.2%) cases (high expression); **Figure 1a**.

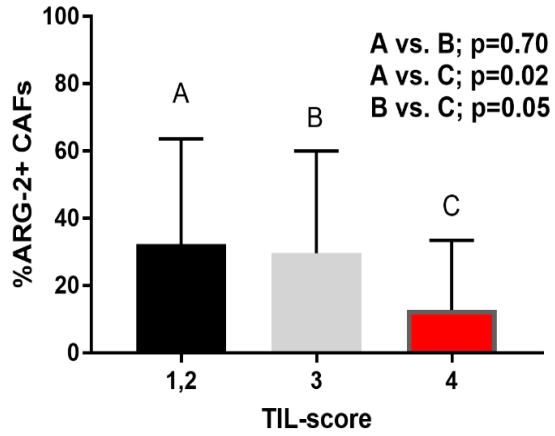
ARG-2 was expressed by stroma cancer associated fibroblasts (CAFs) in 58/98 cases. In 31/98 (31.6%) ARG-2 covered 10-40% of the stroma area (medium expression) and in the remaining 27/98 (27.6%) covered 50-90% of the stroma area (high expression); **Figure 1b**.

		STAGE		
	1	2	3	p-value
ARG-2				
Stroma CAFs				
Negative/Low (40)	24	6	10	
Medium (31)	13	8	10	0.03*
High (27)	9	8	10	

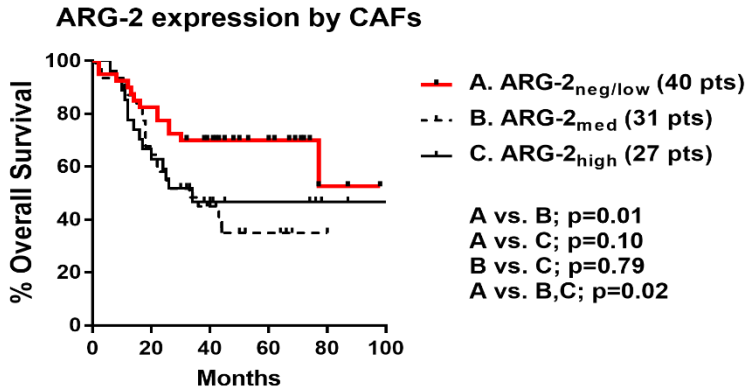
(*) stage 1 vs 2,3 – negative vs. medium/high

ARG-2 expression by cancer cells was not linked with histology or stage.

ARG-2 expression by CAFs was linked with advanced stage (p=0.03).



ARG-2 expression by CAFs was linked with poor TIL-infiltration of tumor stroma (p<0.05).



ARG-2 expression by CAFs was linked with poor disease specific overall survival (p<0.02).

CONCLUSIONS: ARG-2 is expressed by cancer cells and cancer associated fibroblasts (CAFS) in NSCLC. Expression of ARG-2 by CAFs is linked with tumor escape from immune surveillance, local growth and poor survival. ARG-2 blockers may prove of significant therapeutic value for the immunotherapy of NSCLC.