T-4

A 20-years survey by ITA.LI.CA on hepatocellular carcinoma (HCC) in HCV-related cirrhosis

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Background: In the western world HCV is the leading etiologic factor of HCC. Overall HCV impact on HCC-related mortality is increasing, but recent data from Italy suggest an initial drop.
Aims: To evaluate epidemiology, clinical features and survival of HCV-related HCC in a wide time range in Italy.
Methods: Multicenter retrospective study including 2769 patients prospectively recruited by the ITA.LI.CA group. The patients were classified in 3 groups (Group A [G.A.], HCV associated or not with other etiologies, Group B [G.B.], pure HCV, and Group C [G.C.], alternative etiologies) and sub-grouped in 5 years time cohorts (1986-90, 91-95, 96-2000, 2001-2005) but for the last one (2006-2008). Age, gender, Child-Pugh status, diagnosis for surveillance, stage, thrombosis and metastases, treatment and survival (Kaplan-Meier, Log-Rank) were analyzed.
Results: 1780 patients were included in G.A., 1430 in G.B., 989 in G.C. The number of G.A. and G.B. patients reached a peak in the 1996-2000 to then gradually drop, lowest values being observed in the last three years (p=0.0001 G.A., 0.01 G.B.). Mean age at diagnosis progressively increased in G.A. ($p<0.0001$) and G.B. ($p<0.001$), but not in G.C, as did the percentage of cases diagnosed under surveillance (G.A. p=0.019, G.B. p=0.0038) and the share of patients in Child-Pugh A stage (G.A. p=0.01, G.B. p=0.007). Tumor size decreased in all subgroups, stage improved significantly only in G.B. Median survival significantly increased in all groups but more significantly in G.A. and G.B., with a significant difference among G.A. and G.B. vs G.C. in the last 3 time periods (p=0.02).
Conclusions: The incidence of HCC in HCV-related liver disease is decreasing in Italy since 2001. HCV-related HCC patients are older, more frequently diagnosed under surveillance, more frequently characterized by conserved liver function and smaller tumors, with an earlier tumor stage when HCV is the only etiologic factor. Finally their survival dramatically improved in the last 15 years, more than in patients with different etiology. We therefore expect a further drop in incidence and mortality for the disease in Italy in the years to come.

T-5

Oncostatin M, overexpressed in hepatocellular carcinoma, up-regulates SERPIN-B3 expression in hepatic cancer cells

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Background/Aims: SERPINB3 (S-B3), a serine protease inhibitor overexpressed in hepatocellular carcinoma (HCC) and up-regulated by hypoxia through a redox- and HIF2α-dependent mechanism, can stimulate EMT and increased invasiveness in hepatic cancer cells. Along these lines, oncostatin M (OSM) has been proposed to modulate hypoxia-dependent processes such as liver development, regeneration and angiogenesis. Moreover, OSM-related signaling pathway has been recently reported to operate by up-regulating HIF1α. In the present we investigated whether OSM is expressed in human HCC and whether it may be able to up-regulate S-B3 expression in human hepatic cancer cells.
Methods: OSM and S-B3 expression has been investigated by immunohistochemistry on liver specimens from HCV cirrhotic patients carrying G1 and G2 HCC. S-B3 expression has been investigated by employing morphological, molecular and cell biology techniques in HepG2 and HuH7 cells exposed to human recombinant OSM.
Results: In vivo analysis of OSM expression revealed that the cytokine was expressed not only in cirrhotic tissue (as already shown by others) but also in cancer cells of both G1 and G2 HCC. In particular, OSM-positive staining in HCC was detected in some CD68 positive macrophages but mainly in epithelial tumour cells of areas that were also positive for either HIF2α and S-B3. Exposure of human hepatic cancer cells to recombinant OSM resulted in a significant increase of S-B3 mRNA transcription within the first 24 hrs, followed by increased synthesis of the correspondent proteins at 48 hrs. Similarly to what previously described for hypoxia-dependent up-regulation of S-B3, OSM operated by inducing an early increase in intracellular reactive oxygen species (ROS) and recruitment/stabilization of HIF2α.
Conclusions: OSM is overexpressed "in vivo" in human HCC cells in association to HIF2α and S-B3. OSM and hypoxia, as independent signals, can effectively up-regulate the expression of S-B3 in human cancer cells by activating a common redox- and HIF2α-dependent mechanism.

T-6

Oncostatin M stimulates chemotaxis of human hepatic profibrogenic cells

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Background/Aims: Hepatic myofibroblasts (MFs) can originate from hepatic stellate cells (HSC/MFs), portal fibroblasts or bone marrow-derived mesenchymal stem cells (MSCs) and can migrate towards the site of injury and to align with nascent and established fibrotic septa in response to several polypeptides, hypoxia or reactive oxygen species (ROS). Oncostatin M (OSM) is known to orchestrate hypoxia-modulated hepatic processes (development, regeneration, angiogenesis) involving HIF-1. Here we investigated signaling mechanisms regulating migration of human HSC/MFs and human, MF-like and bone marrow-derived, MSCs in response to oncostatin M.
Methods: Signal transduction was evaluated by integrating cell and molecular biology techniques, whereas non-oriented migration and chemotaxis were assessed by wound healing assay and the modified Boyden's chambers assay, respectively. Morphological analysis was performed by immunohistochemistry (IHC) on liver specimens from HCV cirrhotic patients (Metavir F4).
Results: IHC revealed positive stain for OSM in hepatocytes of cirrhotic nodules, mainly in the proximity of fibrotic septa. Human recombinant OSM stimulates non-oriented migration and chemotaxis in HSC/MFs and MSCs involving the the following common features: (a) early intracellular ROS generation and activation of Ras/Erk JNK1/2 as well as of STAT1 and STAT3 signaling pathways and involvement of hypoxia-inducible factor-1α (HIF-1α); (b) OSM-dependent migration, which was inhibited by apocynin, indicating NADPH-oxidase as a major source of ROS; (c) OSM-dependent motogenic action that, similarly to hypoxia, appeared to be exerted in HSC/MFs and MSCs through a biphasic mechanism requiring early generation of ROS and late HIF1-dependent expression and release of VEGF.
Conclusions: OSM may contribute to fibrogenesis by stimulating directional migration of human hepatic MFs through a biphasic, redox- and HIF-1α/VEGF-dependent mechanism.