Methods: We studied 141 cases of early staged non-small cell lung cancer were stained for PD-ECGF (P-GF.44C MoAb) and vascular grade (JC70 MoAb).

Results: Cancer cell PD-ECGF overexpression related to high vascular grade ($p = 0.02$). Early steps of PD-ECGF activation could be identified in 26 cases, where one or two small foci of PD-ECGF overexpression occurred within a general pattern of negative/weak staining. 32 foci of overexpression were analysed as for the local degree of angiogenesis (Chalkley score) and local inflammatory (lymphocyte and macrophage) infiltration comparatively with the remaining negative tumour areas. The mean overall Chalkley score was 4.6 (sd 1.79) vs. 5.53 (sd 2.87) in the areas of PD-ECGF overexpression ($p = 0.006$). The mean chalkley score dropped to 3.93 (sd. 1.98) when assessed only in areas with negative PD-ECGF reactivity ($p = 0.002$). Nineteen out of 26 cases had low degree of lymphoplasmocytic infiltration. In 10/19 (54%) high lymphocytic infiltration was observed in the area of PD-ECGF overexpression which was statistically significant ($p = 0.01$).

Conclusions: The present study provides evidence of a direct causative involvement of PD-ECGF in the process of angiogenesis in NSCLC.

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A prospective study on intratumoral microvessel density (IMD), p53-protein expression and prognosis in colorectal cancer

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Purpose: To investigate the correlation between clinical characteristics, IMD, p53 protein expression, CD44v6 expression, basement membrane and disease-free, overall survival and response to chemotherapy in colorectal carcinoma.

Methods: A prospective study was initiated in 01-90 up to 12-94, collecting all clinicopathological information on patients with operable colorectal carcinoma. Patients with Dukes D carcinoma who needed palliative surgery were included. In addition to preoperative clinical and radiological parameters, transfusion needs, standard pathological staging and grading, p53 overexpression was determined by DO7 staining, collagen IV staining and CD44v6 staining, IMD was determined with a CD-31 immunostaining. The microvessel density was determined according to the consensus report [Vermeulen et al., EJC 32A (14)].

Results: Prospective data collection was performed on 330 patients, with complete clinicopathological data and follow-up currently available for 255 patients. Mean IMD in hot spots is 104/×200 field (median 96; SD 36). 57% of CRC overexpressed p53-protein (DO7 + in >10% of tumour cell nuclei). Increasing p53-protein expression was related to higher IMD counts, confirming our previous results (Microvac Res 1996, 51). 54% of p53 positive CRC showed an IMD > 100 as compared to 28% of the p53 negative group. An inverse association between IMD and CD44v6 expression was observed ($p < 0.05$). In patients with Dukes C and D high IMD predicted for shorter survival.

Conclusion: The mean and median microvessel density in CRC is comparable to IMD reported in breast adenocarcinoma. Angiogenesis counted as IMD in hot spots has prognostic value for disease-free and overall survival in CRC.

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Involvement of Rho proteins in regulation of transformation and apoptosis

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Rho proteins are a family of GTPases belonging to the Ras superfamily, which are critical elements of signal transduction pathways leading to a variety of cellular responses. This family of small GTPases have been involved in diverse biological functions such as cytoskeleton organization, cell growth and transformation, cell motility, migration, metastasis, and response to stress. We have recently demonstrated that Rho proteins are dual regulators of transformation and apoptosis, since overexpression of the *Aplysia californica rho* gene in NIH3T3 cells triggers apoptosis after serum deprivation. Here we report that human Rho proteins such as RhoA, RhoC and Rac1, are capable of inducing apoptosis in different cell systems like murine NIH3T3 cells and human K562 cells. Since K562 cells are devoid of p53, apoptosis induced by Rho is independent of p53. Rho-dependent apoptosis is mediated by the generation of ceramides in both murine and human cells, and it is drastically inhibited by ectopic expression of Bc12, both under in vitro and in vivo conditions. Furthermore, the human oncogenes *vav* and *ost* that have been shown to function as guanine exchange factors for Rho proteins, also were able to induce apoptosis under similar conditions. Finally, the levels of endogenous Rho proteins are increased when cells are exposed to apoptosis-inducing conditions. These results suggest that Rho proteins play an important role in the physiological regulation of apoptosis.

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PCR based detection of melanoma cells: Molecular staging?

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Tyrosinase PCR is a powerful diagnostic procedure to detect circulating melanoma cells and submicroscopic tissue involvement. Since PCR methods are very sensitive for contamination and the methods differ among laboratories, standardized methods and quality controls are necessary in order to facilitate comparison of results, which are heterogenous among different laboratories. The most obvious difference is the variable percentage of patients with stage IV melanoma with PCR detection of TYR transcripts, ranging from 34% to 100%, most likely because of differences in sample preparation. A series of patients studied in Heidelberg revealed the following results: In untreated patients circulating melanoma cells were detected in 4 of 13 with localized disease, 3 of 6 with intransit metastases, 11 of 22 with regional lymph node involvement, and 27 of 30 with distant metastases.

A workshop of the EORTC Melanoma Cooperative Group on this topic was therefore held in January 1996. For quality assurance, a series of blinded samples for analysis of tyrosinase mRNA has been distributed among laboratories of the EORTC-MCG. The result of this quality assurance initiative was, that (1) false positive results were rare, (2) 10^2–10^4 SK Mel 28 cells in 10 ml of whole blood were reliably detected, and (3) 10 SK Mel 28 cells in 10 ml of blood were detected in only two thirds of the laboratories.

Conclusion: The detection of circulating melanoma cells by RT-PCR can be a reliable, although the sensitivity differs even among experienced laboratories, mostly because of differences in pre-PCR processing.

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Cell-cycle dependant expression of the proliferation associated Ki-67 antigen

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Purpose: The monoclonal antibody (mab) Mib-1 is widely used in oncology as a marker for proliferation. Mib-1 binds to the Ki-67 antigen (Ki-67 protein) which is expressed only in all active phases of the cell-cycle (G1, S, G2 and M) but is undetectable in quiescent cells (G0-phase). The structure of the antigen has been shown to be unique: it contains 16 repeats of 122 aa, each of them containing a highly conserved 20 aa "Ki-67 motif" harboring the epitopes for the mabs Mib-1 and Ki-67. We investigated the function of this molecule depending on the cell-cycle phases.

Methods: HeLa cell-culture cells were transiently transfected by a regulated vector-system expressing partial structures of the Ki-67 protein in sense or antisense orientation. The transfected cells were analyzed by FACS, BrdU-incorporation assay, PCR and immunohistochemistry using Mib-1.

Results: The expression of antisense-mRNA specific for the translated 5'region of the Ki-67 protein mRNA caused a decreased expression of the Ki-67 protein, a reduction of S-phase cells and a diminished BrdU-incorporation. Surprisingly, overexpression of three Ki-67 repeats caused similar results. Overexpression of three Ki-67 repeats with an additional SV40-nuclear localization site (NLS) caused an accumulation of cells in G2/M-phase. In the latter cells the mab Mib-1 was unable to detect the antigen in the nuclei of the transfected cells.

Conclusion: We hypothesize that overexpression of the three Ki-67 repeats in the cytoplasm hampers the transport of the native protein into the nuclei by formation of supra molecular complexes. Expression in the nuclei blocks the binding of the mabs and leads to a functional inhibition of the Ki-67 protein in G2/M by protein-protein interactions or by a dominant negative effect.