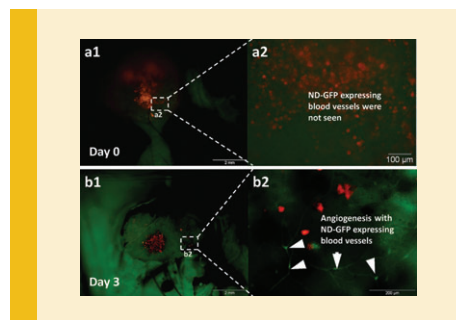


Color-Coded Fluorescence Imaging of Lymph-Node Metastasis, Angiogenesis, and Its Drug-Induced Inhibition

457

Ryoichi Aki, Yasuyuki Amoh, Michael Bouvet, Kensei Katsuoka, and Robert M. Hoffman

ACCEPTED MANUSCRIPT ONLINE 21 SEPTEMBER 2013



Lymph nodes are often the first target of metastatic cancer which can then remetastasize to distant organs. The progression of lymph node metastasis is dependent on sufficient blood supply provided by angiogenesis. In the present study, a color-coded imaging model was developed in order to visualize angiogenesis of lymph node metastasis using green fluorescent protein (GFP) and red fluorescent protein (RFP). Transgenic mice carrying GFP under the control of the nestin second-intron enhancer (ND-GFP mice) were used as hosts. Nascent blood vessels express GFP in these mice. B16F10-RFP melanoma cells were injected into the efferent lymph vessel of the inguinal lymph node of the ND-GFP nude mice, whereby the melanoma cells trafficked to the axillary lymph node. Three days after melanoma implantation, ND-GFP-expressing nascent blood vessels were imaged in the axillary lymph nodes. Seven days after implantation, ND-GFP-expressing nascent blood vessels formed a network in the lymph nodes. ND-GFP-positive

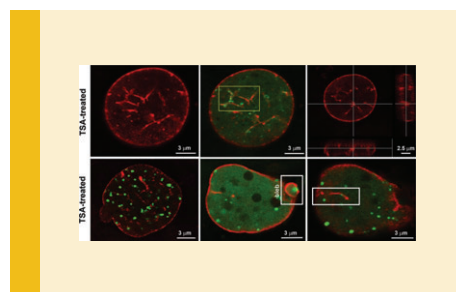
blood vessels surrounded the tumor mass by 14 days after implantation. However, by 28 days after implantation, ND-GFP expression was diminished as the blood vessels matured. Treatment with doxorubicin significantly decreased the mean nascent blood vessel length per tumor volume. These results show that the dual-color ND-GFP blood vessels/RFP-tumor model is a powerful tool to visualize and quantitate angiogenesis of metastatic lymph nodes as well as for evaluation of its inhibition.

Nuclear Structures Surrounding Internal Lamin Invaginations

476

Soňa Legartová, Lenka Stixová, Oskar Laur, Stanislav Kozubek, Petra Sehnalová, and Eva Bártoová

ACCEPTED MANUSCRIPT ONLINE 7 OCTOBER 2013



A- and C-type lamins are intermediate filament proteins responsible for the maintenance of nuclear shape and most likely nuclear architecture. It is proposed that pronounced invaginations of A/C-type lamins into the nuclear interior represent channels for the transport of regulatory molecules to and from nuclear and nucleolar regions. Using fluorescent protein technology and immunofluorescence, it is shown that A-type lamin channels interact with several nuclear components, including fibrillarin- and UBF-positive regions of nucleoli, foci of heterochromatin protein 1 β , polycomb group bodies, and genomic regions associated with DNA repair. Similar associations were observed between A/C-type lamin channels and nuclear pores, lamin-associated protein LAP2 α , and promyelocytic leukemia nuclear bodies. Interestingly, regions with high levels of A/C-type lamins had low levels of B-type lamins, and vice versa. These characteristics were observed in primary and im-

mortalized mouse embryonic fibroblasts as well as human and mouse embryonic stem cell colonies exhibiting stem cell-specific lamin positivity. The findings indicate that internal channels formed by nuclear lamins likely contribute to normal cellular processes through association with various nuclear and nucleolar structures.