Characterization of three novel cell lines established from human metastatic midgut carcinoid

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Background and Aims: Our focus is the cultivation of human midgut carcinoids which are neuroendocrine tumors (NET) derived from enterochromaffin cells (EC) of the embryonic neural crest. Recently, we could establish and characterize three novel cell lines from a human metastatic midgut carcinoid [1]. For the first time we have the potential to study differences in biological characteristics of three cell lines derived from the primary tumor (P-STS), from a lymph node metastasis (L-STS) and from a hepatic metastasis (H-STS) of one patient. Furthermore, the cell lines may serve as a basis to study possible differences in the treatment of primary tumors versus metastases. Since production of biogenic amines, such as serotonin, is a typical feature of midgut carcinoids, we aimed to determine the precise location and quantification of 5-HT using immunogold to elucidate a basic biological difference between the primary tumor and the metastases.
Methods: For immunogold electron microscopic screening of 5-HT, cells were fixed in glutaraldehyde and then labelled using a pre-embedding protocol. The protocol comprised incubating the cells with the primary antibody (polyclonal rabbit-anti-human-serotonin, Chemicon), followed by an incubation with the secondary antibody (1.4nm gold-conjugated goat anti-rabbit antibody; Nanoprobes), a silver enhancement step using IntenSEM (AuroProbe, GE Healthcare), and a gold toning step [2, 3]. Then the cells were postfixed in glutaraldehyde, followed by an osmium tetroxide fixation step, dehydration and embedding in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a Zeiss EM 902 transmission electron microscope (TEM). Gold particles were counted in 30 cells of each cell line (P-STS, L-STS, H-STS). As positive control, the serotonin-producing carcinoid cell line KRJ-I [4] was analysed.
Results and Conclusion:
So far, the ability to study potential therapeutic agents was limited, because only few cell lines from human midgut carcinoids had been available. Recently, three novel continuously growing cell lines were charactericed by immunocytochemical examination and cytogenetic analyses, which confirmed the preservation of neuroendocrine differentiation. Furthermore, the three cell lines were tumorigenous in SCID–mice. In our study we were able to show significant biological differences in the primary tumor (P-STS) versus the metastases (L-STS and H-STS) by 5-HT-quantification using immunogold (Fig. 2 and Fig. 3).Our study should lead to a better understanding of the properties of the primary tumor and the metastases of human midgut carcinoids. Our novel cell lines may serve as preclinical models to develop

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anticancer agents in a malignant disease, where so far the only current curative treatment option is early surgery.

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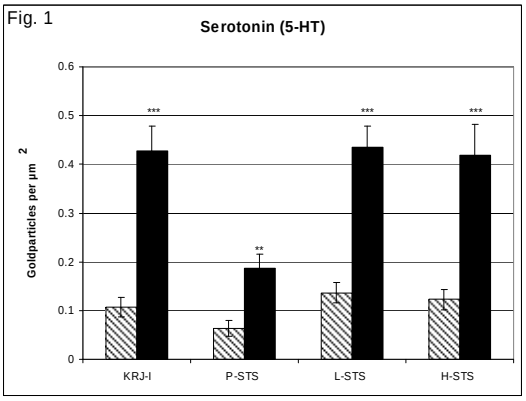

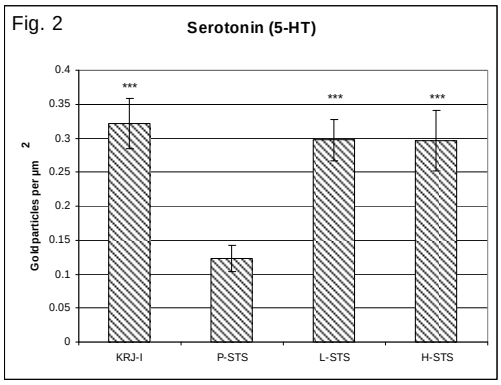

Figure 1. Determination of labelling densities revealed a significant difference (** $p < 0{,}001$; *** $p < 0{,}0001$) between negative controls (hatched) and cells incubated with anti-serotonin antibody (black) (mean ± SEM).
Figure 2. Determination of labelling densities revealed a significant difference (*** $p < 0{,}0001$) between the primary tumor (P-STS) versus the metastases (L-STS and H-STS). KRJ-I, a carcinoid cell line which was characterized previously, for the content of serotonin [4] served as positive control (mean ± SEM).

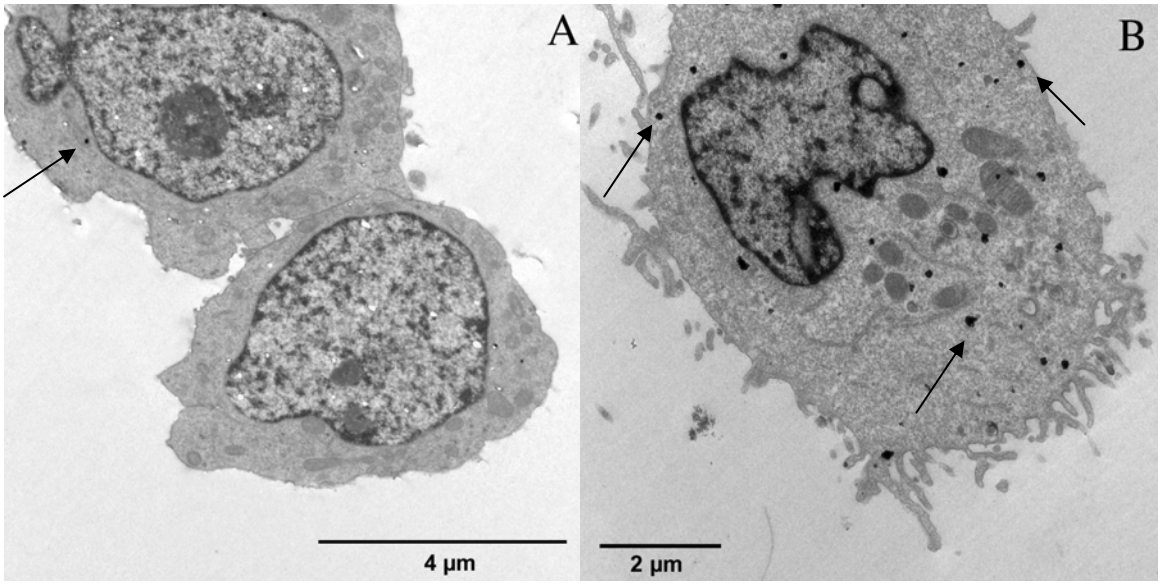

Figure 3. Immunogold electron microscopic demonstration of serotonin (arrows) in the **(A)** primary tumor cell line (P-STS) with low labelling density versus **(B)** a metastasis (L-STS) with high labelling density.