Using Confocal time-lapse Imaging and Superresolution Microscopy to reveal Localization Patterns of the proto-oncogenic Protein DEK

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KEY WORDS: Proto-oncogen DEK, time-lapse Imaging, Structured Illumination Microscopy, subnuclear Localization Patterns.

DEK is a highly conserved and unique non-histone chromatin associated protein overexpressed in several tumor types. Lack of DEK has been shown to sensitize cells to replication stress [1], suggesting a protective role on replication fork progression during S-phase. Based upon these data it was proposed that DEK’s cancerogenic properties might be explained in the context of an oncogene-induced replication stress model. This model is based on the observation that tumor tissue bears signs of DNA replication stress already at very early stages. For developing to full malignancy, tumor cells need to overcome the barrier imposed by the DNA damage response. Once cells have successfully escaped these safeguard mechanisms, DEK would serve as a key factor helping to cope with replication-associated DNA damage, contributing to genomic re-stabilization and supporting cancer "fitness".

Recently, DEK’s localization in transformed cells was compared to non-transformed fibroblasts [2]. In the transformed cell lines DEK was constitutively associated with chromatin throughout the cell cycle, which confirms previous studies with tumor cells [3]. In contrast, in non-transformed cells DEK was absent from early stage mitotic chromatin. Interestingly, when DEK was overexpressed up to levels of tumor cell lines, reconstitution of DEK on mitotic chromatin was accompanied by an increased occurrence of hallmarks of genomic instability. Taken together, these findings suggest that deregulation of DEK drives tumor development not only through replication-associated mechanisms but also by interfering with mitosis.

Here, we investigate DEK’s subnuclear localization with two different approaches: First, we will use an eGFP-DEK knock-in tumor cell line to study DEK’s localization in transformed cells throughout the cell cycle by confocal time-lapse imaging. S-phase specific DEK patterns will be discriminated from those of interphase cells by co-expressing replication fork protein RFP-PCNA. Second, 3D structured illumination microscopy (3D-SIM) will be used to visualize DEK at very high resolution. Emphasis will be laid on the colocalization of immuno-labeled DEK and PCNA both in cancer cells and in fibroblasts to better understand the previously reported differences of DEK localization between transformed and non-transformed cells.