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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A3.33

Enhanced migration and proliferation of human melanoma cells by nonessential amino acid proline

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Background: In the tumour microenvironment, the rewiring of metabolism in response to oncogenic stimuli is recognized as an adaption mechanism to cope with hypoxia, acidosis and cellular stress. Interestingly, amino acids substitute as energy source, feed lipid biosynthesis and represent part of the secretome of tumour cells. We have recently determined the amino-acid composition of conditional media of melanoma cell lines from different disease stages and used multivariate data analysis to identify principle components of the amino-acid profile responsible for growth differences [1]. Proline was found to be taken up by early-stage melanoma cells. Intracellularly, proline is synthesized from either glutamate or ornithine, but alternatively, extracellular proteolysis of collagen in the tumour microenvironment may substitute as another source for proline. However, the biological role of extracellular proline in defined melanoma cell lines remains to be elucidated.
Methods: Human melanoma cells lines reflecting early radial growth phase (WM35) and advanced metastatic stage (A375) were studied for proliferation, migration in scratch assays and proline uptake experiments under a confocal laser scanning microscope. The two cell lines contain the $BRAF^{V600E}$ mutation. Cells were exposed to proline, dansylproline, vemurafenib or the PI3K inhibitor LY294002. Treatments were analysed for multiple comparison with ANOVA and *post hoc* Student–Newman–Keuls test for statistical significance ($p < 0.05$).
Results: The fluorescent dansylproline was readily taken up into WM35 cells reaching a saturation plateau after 40 min. Dansylproline distributed over the cytosol and accumulated in the perinuclear area. Increasing concentrations of unmodified proline significantly enhanced proliferation of WM35 cells, while metastatic A375 cells were virtually unaffected. In gap-closure experiments with WM35 cells, proline significantly enhanced migration into the cell-free area. Co-administration of the PI3K inhibitor LY294002 partially reversed growth advantage in the presence of proline. Conversely, the $BRAF^{V600E}$ inhibitor vemurafenib fully reversed the proline effects. However, vemurafenib lead to morphological changes indicating pro-apoptotic mechanisms.
Discussion: These data confirm a microenvironmental signalling role for the specific amino acid proline, including functional consequences on proliferation and migration independent of the $BRAF^{V600E}$ mutation. Moreover, usage of the fluorescent proline analogue allows screening for amino-acid uptake inhibitors and thereby possible therapeutic intervention.
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Keywords: melanoma – microenvironment – amino-acid signalling – proline

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