These research projects were undertaken in partial fulfillment of the requirements for the MD degree at Sackler Faculty of Medicine, Tel Aviv University in 2017–2018. They were considered the most outstanding of the graduating class

Upregulation of the Unfolded Protein Response: A Novel Activity of Klotho in Colorectal Cancer

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Background: Klotho is a transmembrane protein that can be found in the circulatory system and act as a hormone. In the past, klotho has emerged as a potent tumor suppressor in a wide array of malignancies, including colorectal cancer (CRC). Internal cleavage of klotho forms KL1, which shares with the full-length protein the tumor suppressor activity. Several mechanisms of klotho have been described, including inhibition of the IGF-1 and WNT pathways. However, our preliminary data indicate that inhibition of these pathways can only partially explain the tumor suppressor activity of klotho in CRC. Thus, currently the precise mechanism of klotho in cancer, including in CRC, has not been resolved.

Objectives: To identify the mechanisms mediating the anti-cancer activity of klotho in CRC.

Methods: We transfected different CRC cell lines with expression plasmids encoding klotho, KL1, or pcDNA3 as a control. We used colony and MTT assays to evaluate proliferation and viability of CRC cells. To identify the unique gene signature of klotho, we performed a gene expression array and validated the expression pattern of specific genes using qRT-PCR.

Results: Overexpression with klotho or KL1 both inhibited colony formation and reduced the viability of CRC cells. The gene expression array revealed that both klotho and KL1 overexpression enhanced the unfolded protein response (UPR) and qRT-PCR validated these findings. The UPR is a set of signaling pathways, which evolves when endoplasmic reticulum (ER) stress occurs in the cell. Cancer cells relay on UPR for their survival, but unresolved, prolonged ER stress can lead to UPR-mediated apoptosis. Klotho upregulated the pro-apoptotic pathways of the UPR and attenuation of the UPR, using tauroursodeoxycholic acid (TUDCA), abolished this increase. Importantly, TUDCA also partially abrogated klotho tumor suppressor activity in CRC.

Conclusions: This study shows klotho as a tumor suppressor in CRC. It identifies, for the first time, the UPR as a pathway mediating the effect of klotho in cancer. These results suggest that administration of exogenous klotho or KL1 may serve as a novel strategy for the prevention and treatment of CRC.

*This work was conducted as part of a PhD project

Combination of Staphylolysin (LasA) with Vancomycin in the Management of Staphylococcal Endophthalmitis

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Background: Staphylococcus aureus (*S. aureus*) endophthalmitis therapy is increasingly challenging due to the emergence of strains resistant to vancomycin, the preferred treatment today; therefore, new treatments are needed. Staphylolysin (also called LasA protease), a staphylolytic endopeptidase secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*), causes lysis of *S. aureus* cells. Its action is not affected by the strain, which may overcome the problem of resistance to antibiotics.

Objectives: To evaluate the effect of the combination of LasA protease and vancomycin as a therapy for experimental methicillin resistant *S. Aureus* (MRSA) endophthalmitis.

Methods: Endophthalmitis was induced in the right eyes of 48 rats by an intravitreal injection of 450 MRSA cells. Six hours later, treatment with intravitreal injections was evaluated in the following four groups: LasA, vancomycin, LasA and vancomycin combined, and a control group (vehicle alone). The rats were sacrificed 48 hours after infection, and the vitreous was withdrawn for counting of colony forming units (CFU). Two experiments were conducted using different LasA and vancomycin concentrations. In the first experiment the concentrations were 0.5 mg/ml LasA and 10 mg/ml vancomycin. In the second experiment, the concentrations of both were reduced by half (0.25 mg/ml LasA and 5 mg/ml vancomycin).

Results: Combination therapy significantly reduced the median CFU in both experiments as compared to controls. In the first experiment, CFU/vitreous for LasA and vancomycin combined

and for the control were 0–200 and 83,000 (7,700–220,000), respectively (P = 0.042). In the second experiment, CFU/vitreous for LasA and vancomycin combined and the control were 0 and 6,000 (1,000–60,000), respectively (P = 0.006).

Conclusions: Combined treatment of LasA and vancomycin is an effective treatment for staphylococcal endophthalmitis. These results may be the basis of a new innovative treatment for endophthalmitis caused by MRSA.

Characterizing Melanoma Cell Dissemination Patterns in a Novel Mouse Model of Spontaneous Metastasis

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Background: Malignant melanoma is the deadliest of all skin cancers because it is highly metastatic. Metastatic melanoma is still mostly incurable. The interactions between the tumor cells and the microenvironment that enable the homing and growth of metastases to different organs are poorly characterized.

Objectives: To establish a novel model of spontaneous melanoma metastasis in immunocompetent mice. To provide a platform to study the occurrence of micrometastases to elucidate the earliest steps in the metastatic process.

Methods: We analyzed the presence of micrometastases by qRT-PCR in brains, lungs, and liver from four cohorts of mice. We utilized transwell migration experiments in vitro to gain mechanistic insights. Finally, we compared the metastatic spread in the murine model to data of human melanoma metastasis.

Results: We found that the liver was the most common site for micrometastases followed by the lungs and the brain. Moreover, there was no significant association between the occurrences of metastases in the different organs. We analyzed the metastatic spread pattern of BT-RMS, a melanoma cell variant that we isolated from brain metastases. When subdermally re-injected, BT-RMS cells showed higher tropism toward the brain in vivo. In vitro, melanoma cells migrated significantly toward astrocytes, but soluble factors extracted from normal brains inhibited their migration. Importantly, comparison of the occurrence of metastases in our model to postmortem clinical studies revealed a striking resemblance between the findings.

Conclusions: Our immunocompetent mouse model showed a clear resemblance to the natural history of the human melanoma disease. Therefore, using this model for studying the role of the microenvironment in metastatic spread as well as the molecular mechanisms that govern early metastatic growth might aid in developing novel therapeutic approaches that may prevent metastatic occurrence and relapse.

Erratum: In the article "Correlations Between Core Needle Biopsy and Excisional Biopsy Findings in Suspected Breast Lesions: A Single Center Study" by Davidson et al. that appeared in the July 2018 issue of *IMAJ* on page 401, an error occurred in Table 2. The correct table appears in the online version of the article.

Capsule

Bacterial factors that predict relapse after tuberculosis therapy

Approximately 5% of patients with drug-susceptible tuberculosis have a relapse after 6 months of first-line therapy, as do approximately 20% of patients after 4 months of short-course therapy. **Colangeli** and colleagues postulated that by analyzing pretreatment isolates of *Mycobacterium tuberculosis* obtained from patients who subsequently had a relapse or were cured, clinicians could determine any correlations between the minimum inhibitory concentration (MIC) of a drug below the standard resistance breakpoint and the relapse risk after treatment. In the development cohort, the mean ± SD MIC of isoniazid below the breakpoint was 0.0334 ± 0.0085 µg/ml in the relapse group and 0.0286 ± 0.0092 µg/ml in the cure group. This finding represented a higher value in the relapse group by a factor of 1.17 (P = 0.02). The corresponding MIC values of rifampin were 0.0695 ± 0.0276 and 0.0453 ± 0.0223 µg/ml, respectively, which represented a higher value in the relapse group by a factor of 1.53 (P < 0.001). Higher MIC values remained associated with relapse in a multivariable analysis that included other significant between-group differences. In an analysis of receiver-operating-characteristic curves of relapse based on these MIC values, the area under the curve (AUC) was 0.779. In the development cohort, the AUC in a multivariable model that included MIC values was 0.875. In the validation cohort, the MIC values either alone or combined with other patient characteristics were also predictive of relapse, with AUC values of 0.964 and 0.929, respectively. The use of a model score for the MIC values of isoniazid and rifampin to achieve 75.0% sensitivity in cross-validation analysis predicted relapse with a specificity of 76.5% in the development cohort and a sensitivity of 70.0% and a specificity of 100% in the validation cohort.

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